

APPLICATION  
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TITLE: 26583, A NOVEL SERINE/THREONINE PHOSPHATASE  
AND USES THEREFOR

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## 26583, A NOVEL SERINE/THREONINE PHOSPHATASE AND USES THEREFOR

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### CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. provisional application number 60/187,454, filed on March 7, 2000, the contents of which are incorporated herein by reference.

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### BACKGROUND OF THE INVENTION

Protein phosphatases are enzymes that reverse the actions of protein kinases by cleaving phosphate from serine, threonine, and/or tyrosine residues in proteins. The cellular roles of protein phosphatases are many and diverse. The protein phosphatases are divided into three groups according to catalytic function: (1) protein phosphatases that dephosphorylate serine and threonine residues; (2) protein phosphatases which dephosphorylate tyrosine residues; and (3) protein phosphatases which dephosphorylate serine, threonine and tyrosine residues.

Serine/threonine protein phosphatases are associated with the regulation of cholesterol biosynthesis, glycogen metabolism, muscle contractility, calcium ion channels, protein synthesis, regulation of the G2 to M transition of the cell cycle, regulation of glycolysis (6-phosphofructo-2-kinase and pyruvate kinase), glycogenolysis (phosphorylase kinase subunit), gluconeogenesis (fructose-2,6-bisphosphatase and pyruvate kinase), amino-acid degradation (phenylalanine hydroxylase), lipid metabolism (acetyl-CoA carboxylase), catecholamine synthesis (tyrosine hydroxylase) and protein synthesis (elongation factor 2).

Protein tyrosine phosphatases (PTPs) are a family of intracellular and integral membrane phosphatases that dephosphorylate tyrosine residues in proteins. PTPs have been identified in mammals, *Drosophila* and *Schiz. pombe* and are implicated in the control of normal and neoplastic growth and proliferation. They have also been found encoded by plasmids in bacteria of the genus *Yersinia*, where they are implicated in pathogenicity.

Phosphatases which dephosphorylate serine and threonine residues as well as tyrosine residues (PT/SPS) are distantly related in sequence to the protein tyrosine phosphatases. These phosphatases have been found in mammals, *Xenopus*, *Drosophila* and yeasts, where they are essential for cell division, and in vaccinia virus, where they may be involved in pathogenicity.

Generally, the balance of protein phosphorylation in a cell depends on the level of protein kinase and protein phosphatase activity. Protein phosphorylation is important for the regulation of numerous metabolic processes such as cholesterol biosynthesis and has been associated with cell cycle progression and transformation of cells. Thus, protein phosphatases can serve as positive or negative regulators of metabolic function as well as cell growth and differentiation. Given the important biological roles and properties of phosphatases, there exists a need for the identification of novel genes encoding such proteins as well as for the discovery of modulators of such molecules for use in regulating a variety of normal and/or pathological cellular processes.

#### SUMMARY OF THE INVENTION

The present invention is based, in part, on the discovery of a novel gene predicted to encode a serine/threonine phosphatase, referred to herein as "26583."

The nucleotide sequence of a cDNA encoding 26583 is shown in SEQ ID NO:1, and the amino acid sequence of a 26583 polypeptide is shown in SEQ ID NO:2. In addition, the nucleotide sequence of the coding region is depicted in SEQ ID NO:3.

Accordingly, in one aspect, the invention features a nucleic acid molecule that encodes a 26583 protein or polypeptide, e.g., a biologically active portion of the 26583 protein. In a preferred embodiment, the isolated nucleic acid molecule encodes a polypeptide having the amino acid sequence of SEQ ID NO:2. In other embodiments, the invention provides isolated 26583 nucleic acid molecules having the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, or the sequence of the DNA insert of the plasmid deposited with ATCC Accession Number \_\_\_\_\_. In still other embodiments, the invention provides nucleic acid molecules that are substantially identical (e.g., naturally occurring allelic variants) to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, or the sequence of the DNA insert of the plasmid deposited with ATCC Accession Number \_\_\_\_\_. In other

embodiments, the invention provides a nucleic acid molecule that hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or 3, or the sequence of the DNA insert of the plasmid deposited with ATCC Accession Number \_\_\_\_, wherein the nucleic acid  
5 encodes a full length 26583 protein or an active fragment thereof.

In a related aspect, the invention further provides nucleic acid constructs that include a 26583 nucleic acid molecule described herein. In certain embodiments, the nucleic acid molecules of the invention are operatively linked to native or heterologous regulatory sequences. Also included are vectors and host cells  
10 containing the 26583 nucleic acid molecules of the invention, e.g., vectors and host cells suitable for producing 26583 nucleic acid molecules and polypeptides.

In another related aspect, the invention provides nucleic acid fragments suitable as primers or hybridization probes for the detection of 26583-encoding nucleic acids.

15 In still another related aspect, isolated nucleic acid molecules that are antisense to a 26583 encoding nucleic acid molecule are provided.

In another aspect, the invention features 26583 polypeptides and biologically active or antigenic fragments thereof that are useful, e.g., as reagents or targets in assays applicable to treatment and diagnosis of 26583-mediated or related disorders.  
20 In another embodiment, the invention provides 26583 polypeptides having a 26583 activity. Preferred polypeptides are 26583 proteins including at least one phosphatase catalytic domain, and, preferably, having a 26583 activity, e.g., a 26583 activity as described herein.

In other embodiments, the invention provides 26583 polypeptides, e.g., a  
25 26583 polypeptide having the amino acid sequence shown in SEQ ID NO:2; the amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC Accession Number \_\_\_\_; an amino acid sequence that is substantially identical to the amino acid sequence shown in SEQ ID NO:2; or an amino acid sequence encoded by a nucleic acid molecule having a nucleotide sequence that hybridizes  
30 under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or 3, or the sequence of the DNA insert of the plasmid deposited with ATCC Accession Number \_\_\_\_, wherein the nucleic acid encodes a full length 26583 protein or an active fragment thereof.

In a related aspect, the invention provides 26583 polypeptides or fragments operatively linked to non-26583 polypeptides to form fusion proteins.

In another aspect, the invention features antibodies and antigen-binding fragments thereof, that react with, or more preferably specifically bind 26583 polypeptides.

In another aspect, the invention provides methods of screening for compounds that modulate the expression or activity of the 26583 polypeptides or nucleic acids.

In still another aspect, the invention provides a process for modulating 26583 polypeptide or nucleic acid expression or activity, e.g. using the screened compounds.

In certain embodiments, the methods involve treatment of conditions related to decreased activity or expression of the 26583 polypeptides or nucleic acids, such as conditions involving cholesterol biosynthesis, mitochondrial dysfunction, or aberrant cellular proliferation of a 26583 expressing cell, e.g., a lung cell, a breast cell, a colon cell, a liver cell, or a brain cell.

In still another aspect, the invention features a method of modulating (e.g., enhancing or inhibiting) the proliferation, survival, and/or differentiation of a cell, e.g., a 26583-expressing cell, e.g., a lung cell, a breast cell, a colon cell, a liver cell, or a brain cell. The method includes contacting the cell with an agent that modulates the activity or expression of a 26583 polypeptide or nucleic acid, in an amount effective to modulate the proliferation and/or differentiation of the cell.

In a preferred embodiment, the 26583 polypeptide has an amino acid sequence identical to, or substantially identical to, SEQ ID NO:2. In other embodiments, the 26583 polypeptide is a fragment of at least 15, 20, 50, 100, 150, 200, 213, 250, or more contiguous amino acids of SEQ ID NO:2. In a preferred embodiment, the 26583 polypeptide is a fragment of at least 213 contiguous amino acids of SEQ ID NO:2.

In a preferred embodiment, the 26583 nucleic acid has a nucleotide sequence identical to, or substantially identical to, SEQ ID NO:1 or 3. In other embodiments, the 26583 nucleic acid is a fragment of at least 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, or more contiguous nucleotides of SEQ ID NO:1 or 3.

In a preferred embodiment, the agent modulates (e.g., increases or decreases) protein phosphatase activity, e.g., serine/threonine phosphatase activity.

In a preferred embodiment, the agent modulates (e.g., increases or decreases) expression of the 26583 nucleic acid by, e.g., modulating transcription, mRNA stability, etc.

In preferred embodiments, the agent is a peptide, a phosphopeptide, a small molecule, e.g., a member of a combinatorial library, or an antibody, or any combination thereof. The antibody can be conjugated to a therapeutic moiety selected from the group consisting of a cytotoxin, a cytotoxic agent, and a radioactive metal ion.

In additional preferred embodiments, the agent is an antisense, a ribozyme, or a triple helix molecule, or a 26583 nucleic acid, or any combination thereof.

In a preferred embodiment, the agent is administered in combination with a cytotoxic agent.

In a preferred embodiment, the cell, e.g., the 26583-expressing cell, is a lung cell, a breast cell, a colon cell, a liver cell, or a brain cell, e.g., a neuron or glial cell.

In a preferred embodiment, the cell, e.g., the 26583-expressing cell, is a tumor cell, e.g., a lung, breast, colon, liver, or brain tumor cell.

In a preferred embodiment, the cell, e.g., the 26583-expressing cell, is further contacted with a protein, e.g., a cytokine or a hormone. Exemplary proteins include, but are not limited to, G-CSF, GM-CSF, stem cell factor, interleukin-3 (IL-3), IL-4, Flt-3 ligand, thrombopoietin, and erythropoietin. The protein contacting step can occur before, at the same time, or after the agent is contacted. The protein contacting step can be effected *in vitro* or *ex vivo*. For example, the cell, e.g., the 26583-expressing cell is obtained from a subject, e.g., a patient, and contacted with the protein *ex vivo*. The treated cell can be re-introduced into the subject. Alternatively, the protein contacting step can occur *in vivo*.

In a preferred embodiment, the agent and the 26583-polypeptide or nucleic acid are contacted *in vitro* or *ex vivo*.

In a preferred embodiment, the contacting step is effected *in vivo* in a subject, e.g., as part of a therapeutic or prophylactic protocol. Preferably, the subject is a human, e.g., a patient with a metabolic disorder, e.g., a mitochondrial related disorder or a cholesterol biosynthesis related disorder, or a patient with a cell proliferation or differentiation disorder, e.g., a tumor. For example, the subject can be a cancer patient, e.g., a patient with a lung, breast, colon, liver, or brain tumor. The subject

can also be a patient with diabetes mellitus or a neurodegenerative disorder (e.g., Parkinson's, Huntington's, or Alzheimer's disease). In other embodiments, the subject is a non-human animal, e.g., an experimental animal.

The contacting step(s) can be repeated.

5 In a preferred embodiment, the agent decreases the proliferation and/or enhances the differentiation of the cell, e.g., the 26583-expressing cell, e.g., the lung, breast, colon, liver, or brain cell. Such agents can be used to treat or prevent cancers, e.g., liver, breast, brain, colon, or lung carcinomas.

In yet another aspect, the invention features a method of treating or preventing  
10 a disorder, e.g., a metabolic disorder, e.g., a mitochondrial related disorder or a cholesterol biosynthesis related disorder; or a cellular proliferation and/or differentiation disorder, in a subject. The method includes administering to the subject an effective amount of an agent that modulates the activity or expression of a 26583 polypeptide or nucleic acid such that the disorder is ameliorated or prevented.

15 In a preferred embodiment, the 26583 polypeptide has an amino acid sequence identical to, or substantially identical to, SEQ ID NO:2. In other embodiments, the 26583 polypeptide is a fragment of at least 15, 20, 50, 100, 150, or more contiguous amino acids of SEQ ID NO:2. In a preferred embodiment, the 26583 polypeptide is a fragment of at least 213 contiguous amino acids of SEQ ID NO:2.

20 In a preferred embodiment, the 26583 nucleic acid has a nucleotide sequence identical to, or substantially identical to, SEQ ID NO:1 or 3. In other embodiments, the 26583 nucleic acid is a fragment of at least 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, or more contiguous nucleotides of SEQ ID NO:1 or 3.

In a preferred embodiment, the agent modulates (e.g., increases or decreases)  
25 protein phosphatase activity.

In a preferred embodiment, the agent modulates (e.g., increases or decreases) expression of the 26583 nucleic acid by, e.g., modulating transcription, mRNA stability, etc.

In preferred embodiments, the agent is a peptide, a phosphopeptide, a small  
30 molecule, e.g., a member of a combinatorial library, or an antibody, or any combination thereof. The antibody can be conjugated to a therapeutic moiety selected from the group consisting of a cytotoxin, a cytotoxic agent and a radioactive metal ion.

In additional preferred embodiments, the agent is an antisense, a ribozyme, or a triple helix molecule, or an 26583 nucleic acid, or any combination thereof.

In a preferred embodiment, the agent is administered in combination with a cytotoxic agent.

5 In a preferred embodiment, the subject is a human, e.g., a patient with a metabolic disorder, e.g., a mitochondrial related disorder or a cholesterol biosynthesis related disorder, e.g., hypo- or hypercholesterolemia, diabetes mellitus, or a neurodegenerative disorder (e.g., Parkinson's, Huntington's, or Alzheimer's disease). The subject can also be a patient with a cell proliferation or differentiation disorder,  
10 e.g., a tumor, e.g., a patient with a lung, breast, colon, liver, or brain tumor. In other embodiments, the subject is a non-human animal, e.g., an experimental animal.

In a preferred embodiment, the agent decreases the proliferation and/or enhances the differentiation of the cell, e.g., the 26583-expressing cell, e.g., the lung, breast, colon, liver, or brain cell. Such agents can be used to treat or prevent cancers,  
15 e.g., liver, breast, brain, colon, or lung carcinomas.

In a preferred embodiment, the disorder is a metabolic disorder, e.g., a cholesterol synthesis disorder, e.g., hypo- or hypercholesterolemia; or a mitochondrial related disorder, e.g., diabetes mellitus, or Parkinson's, Huntington's, or Alzheimer's disease.

20 In a preferred embodiment, the disorder is a cancer, e.g., a lung, breast, colon, liver, or brain cancer.

In a preferred embodiment, the method further includes administering an effective amount of a protein, e.g., a cytokine or a hormone, to the subject. Exemplary proteins include, but are not limited to, G-CSF, GM-CSF, stem cell factor, interleukin-3 (IL-3), IL-4, Flt-3 ligand, thrombopoietin, and erythropoietin. The  
25 protein can be administered before, at the same time or after, administration of the agent.

The administration of the agent and/or protein can be repeated.

In still another aspect, the invention features a method for evaluating the  
30 efficacy of a treatment of a disorder, in a subject. The method includes treating a subject with a protocol under evaluation; assessing the expression of a 26583 nucleic acid or 26583 polypeptide, such that a change in the level of 26583 nucleic acid or



26583 polypeptide after treatment, relative to the level before treatment, is indicative of the efficacy of the treatment of the disorder.

In a preferred embodiment, the disorder is a metabolic disorder, e.g., a cholesterol synthesis disorder, e.g., hypo- or hypercholesterolemia; or a mitochondrial related disorder, e.g., diabetes mellitus, or Parkinson's, Huntington's, or Alzheimer's disease.

In a preferred embodiment, the disorder is a cancer, e.g., a lung, breast, colon, liver, or brain cancer.

In a preferred embodiment, the subject is a human.

In a preferred embodiment, the subject is an experimental animal, e.g., an animal model for a metabolic disorder or cancer.

In a preferred embodiment, the method can further include treating the subject with a protein, e.g., a cytokine or a hormone. Exemplary proteins include, but are not limited to, G-CSF, GM-CSF, stem cell factor, interleukin-3 (IL-3), IL-4, Flt-3 ligand, thrombopoietin, and erythropoietin.

The invention also features a method of diagnosing a disorder, e.g., a metabolic disorder or a cell proliferation/differentiation disorder, e.g., cancer, in a subject. The method includes evaluating the expression or activity of a 26583 nucleic acid or a 26583 polypeptide, such that, a difference in the level of 26583 nucleic acid or 26583 polypeptide relative to a normal subject or a cohort of normal subjects is indicative of the disorder.

In a preferred embodiment, the subject is a human.

In a preferred embodiment, the evaluating step occurs *in vitro* or *ex vivo*. For example, a sample, e.g., a blood sample, is obtained from the subject.

In a preferred embodiment, the evaluating step occurs *in vivo*. For example, by administering to the subject a detectably labeled agent that interacts with the 26583 nucleic acid or polypeptide, such that a signal is generated relative to the level of activity or expression of the 26583 nucleic acid or polypeptide.

In a preferred embodiment, the disorder is a metabolic disorder, e.g., a cholesterol synthesis disorder, e.g., hypo- or hypercholesterolemia; or a mitochondrial related disorder, e.g., diabetes mellitus, or Parkinson's, Huntington's, or Alzheimer's disease.

In a preferred embodiment, the disorder is a cancer, e.g., a lung, breast, colon, liver, or brain cancer.

The invention also provides assays for determining the activity of or the presence or absence of 26583 polypeptides or nucleic acid molecules in a biological sample, including for disease diagnosis.

In further aspect, the invention provides assays for determining the presence or absence of a genetic alteration in a 26583 polypeptide or nucleic acid molecule, including for disease diagnosis.

In yet another aspect, the invention features a method for identifying an agent, e.g., a compound, which modulates the activity of a 26583 polypeptide, e.g., a 26583 polypeptide as described herein, or the expression of a 26583 nucleic acid, e.g., a 26583 nucleic acid as described herein, including contacting the 26583 polypeptide or nucleic acid with a test agent (e.g., a test compound); and determining the effect of the test compound on the activity of the polypeptide or nucleic acid to thereby identify a compound which modulates the activity of the polypeptide or nucleic acid.

In a preferred embodiment, the activity of the 26583 polypeptide is a protein phosphatase activity.

In a preferred embodiment, the activity of the 26583 polypeptide is proliferation, differentiation, and/or survival of a cell, e.g., a 26583-expressing cell, e.g., a lung, breast, colon, liver, or brain cell.

In preferred embodiments, the agent is a peptide, a phosphopeptide, a small molecule, e.g., a member of a combinatorial library, or an antibody, or any combination thereof.

In additional preferred embodiments, the agent is an antisense, a ribozyme, or a triple helix molecule, or an 26583 nucleic acid, or any combination thereof.

In another aspect, the invention features a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality, and each address of the plurality having a unique capture probe, e.g., a nucleic acid or peptide sequence. At least one address of the plurality has a capture probe that recognizes a 26583 molecule. In one embodiment, the capture probe is a nucleic acid, e.g., a probe complementary to a 26583 nucleic acid sequence. In another embodiment, the capture probe is a polypeptide, e.g., an antibody specific for 26583 polypeptides. Also featured is a

method of analyzing a sample by contacting the sample to the aforementioned array and detecting binding of the sample to the array.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

### BRIEF DESCRIPTION OF THE DRAWINGS

*Figure 1A-B* depicts a cDNA sequence (SEQ ID NO:1) and predicted amino acid sequence (SEQ ID NO:2) of human 26583. The methionine-initiated open reading frame of human 26583 (without the 5' and 3' untranslated regions) starts at nucleotide 462 and ends at nucleotide 2075 of SEQ ID NO:1 (shown also as coding sequence (SEQ ID NO:3)).

*Figure 2* depicts a hydropathy plot of human 26583. Relative hydrophobic residues are shown above the dashed horizontal line, and relative hydrophilic residues are below the dashed horizontal line. The cysteine residues (cys) are indicated by short vertical lines just below the hydropathy trace. The numbers corresponding to the amino acid sequence of human 26583 are indicated. Polypeptides of the invention include fragments which include: all or part of a hydrophobic sequence, i.e., a sequence above the dashed line, e.g., the sequence of 262-279; all or part of a hydrophilic sequence, i.e., a sequence below the dashed line, e.g., the sequence of 60-70; a sequence which includes a Cys, or a glycosylation site.

*Figures 3A and 3B* depict alignments of human 26583 amino acid sequence with a consensus amino acid sequence derived from protein phosphatase 2C (PP2C) (Figure 3A) and protein phosphatase 2C\_4 (PP2C\_4) (Figure 3B). In Figure 3A, the upper sequence is the consensus amino acid sequence (SEQ ID NO:4) for PP2C, while the lower amino acid sequence corresponds to amino acids 173 to 461 of SEQ ID NO:2. In Figure 3B, the upper sequence is the consensus amino acid sequence (SEQ ID NO:5) for PP2C\_4, while the lower amino acid sequence corresponds to amino acids 99 to 522 of SEQ ID NO:2.

*Figure 4* shows a bar graph depicting relative 26583 mRNA expression as determined by TaqMan assays on mRNA derived from the following tissue samples. Columns are numbered at five-column intervals at the bottom of the Figure (i.e., columns 1-42), and correspond to the following: columns 1-3, normal breast;

columns 4-10, breast tumor; columns 11-13, normal lung; columns 14-20, lung tumor; columns 21-23, normal colon; columns 24-31, colon tumor; columns 32-35, colon metastases; columns 36-37, normal liver; columns 38-39, normal brain; columns 40-42, brain tumor.

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### DETAILED DESCRIPTION

The human 26583 sequence (Figure 1; SEQ ID NO:1), which is approximately 2838 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 1613 nucleotides (nucleotides 462 to 2075 of SEQ ID NO:1; SEQ ID NO:3). The coding sequence encodes a 537 amino acid protein (SEQ ID NO:2).

Human 26583 contains the following regions or other structural features: a predicted serine/threonine catalytic domain at residues 172-461; a predicted serine/threonine catalytic domain at residues 99-523; one predicted N-glycosylation site (PS00001) from about amino acids 105 to 108; five predicted Protein Kinase C sites (PS00005) from about amino acids 95 to 97, 156 to 158, 182 to 184, 211 to 213 and 463 to 465 of SEQ ID NO:2; five predicted Casein Kinase II phosphorylation sites (PS00006) from about amino acids 172 to 175, 228 to 231, 371 to 374, 471 to 474 and 505 to 508 of SEQ ID NO:2; seven predicted N-myristoylation sites (PS00008) from about amino acids 137 to 142, 148 to 153, 271 to 276, 303 to 308, 419 to 424, 456 to 461 and 531 to 536 of SEQ ID NO:2; one amidation site (PS00009) at about amino acids 67 to 70 of SEQ ID NO:2; and one protein phosphatase 2C signature (PS01037) from about amino acids 139 to 147 of SEQ ID NO:2.

For general information regarding PFAM identifiers, PS prefix and PF prefix domain identification numbers, refer to Sonnhammer *et al.* (1997) *Protein* 28:405-420 and <http://www.psc.edu/general/software/packages/pfam/pfam.html>.

A plasmid containing the nucleotide sequence encoding human 26583 (clone Fbh26583FL) was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on \_\_\_\_\_ and assigned Accession Number \_\_\_\_\_. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for

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those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

The 26583 protein contains a significant number of structural characteristics in common with members of the serine/threonine phosphatase family. The term “family” when referring to the protein and nucleic acid molecules of the invention means two or more proteins or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide sequence homology as defined herein. Such family members can be naturally or non-naturally occurring and can be from either the same or different species. For example, a family can contain a first protein of human origin as well as other distinct proteins of human origin, or alternatively, can contain homologues of non-human origin, *e.g.*, rat or mouse proteins. Members of a family can also have common functional characteristics.

A 26583 polypeptide of the invention can include a “serine/threonine phosphatase catalytic domain” or regions homologous with a “serine/threonine phosphatase catalytic domain.” As used herein, the term “serine/threonine phosphatase catalytic domain” refers to an amino acid sequence having about 200 to 450, preferably about 150 to 350, more preferably about 100 to 300, and even more preferably about 288 amino acid residues.

Based on structural similarities, members of the serine/threonine phosphatase family have been classified into various subfamilies, including four major types of protein phosphatase catalytic subunits that dephosphorylate serine and threonine residues. These enzymes are termed protein phosphatases 1, 2A, 2B, and 2C (PP1, PP2A, PP2B and PP2C, the human genome symbols being PPP1, PPP2, PPP3 and PPM1 respectively). Protein phosphatase PP1 appears to have pleiotropic actions in the regulation of glycogen metabolism, muscle contractility, calcium ion channels, protein synthesis and cell division. Protein phosphatase 2A (PP2A) dephosphorylates enzymes involved in the regulation of glycolysis (6-phosphofructo-2-kinase and pyruvate kinase), glycogenolysis (phosphorylase kinase subunit), gluconeogenesis (fructose-2,6-bisphosphatase and pyruvate kinase), amino-acid degradation (phenylalanine hydroxylase), lipid metabolism (acetyl-CoA carboxylase), catecholamine synthesis (tyrosine hydroxylase) and protein synthesis (elongation factor 2). The catalytic subunit has also been identified as a negative regulator of the

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signaling mechanisms; (4) modulation of cell proliferation or growth; (5) modulation of cell differentiation; (6) modulation of cell survival; (7) modulation of transformation; (8) modulation of apoptosis of a cell (*e.g.*, a cancer cell); (9) modulation of cholesterol biosynthesis; (10) modulation of glycogen metabolism; (11) modulation of muscle contractility; (12) modulation of calcium ion channel activity; (13) modulation of glycolysis, glycogenolysis, or gluconeogenesis; (14) modulation of amino-acid degradation; (15) modulation of lipid metabolism; and/or (16) modulation of catecholamine synthesis.

In a preferred embodiment, a 26583 polypeptide or protein has a “serine/threonine phosphatase catalytic domain” refers to an amino acid sequence having about 200 to 450, preferably about 150 to 350, more preferably about 100 to 300, and even more preferably about 288 amino acid residues and has at least about 70% 80% 90% 95%, 99%, or 100% homology with a “serine/threonine phosphatase catalytic domain,” *e.g.*, the serine/threonine phosphatase catalytic domain of human 26583.

Thus, a 26583 molecule of the present invention can be identified based on the presence of a “serine/threonine phosphatase catalytic domain” in the protein or corresponding nucleic acid molecule. Preferably, a serine/threonine phosphatase catalytic domain includes a protein domain having an amino acid sequence of about 200 to 500 amino acid residues and having a bit score for the alignment of the sequence to the fibroblast growth factor domain (HMM) of at least 150. Preferably, a “serine/threonine phosphatase catalytic domain” refers to an amino acid sequence having about 200 to 500, preferably about 250 to 400, more preferably about 250 to 350 amino acid residues and has a bit score for the alignment of the sequence to a serine/threonine phosphatase catalytic domain (HMM) of at least 100, 200, 250 or greater. An alignment of the serine/threonine phosphatase of human 26583 (SEQ ID NO:2) with a consensus amino acid sequence derived from a hidden Markov model is depicted in Figures 3A-3B.

To identify the presence of a “serine/threonine phosphatase catalytic domain” in a 26583 protein sequence and to make the determination that a polypeptide or protein of interest has a particular profile, the amino acid sequence of the protein can be searched against a database of HMMs (*e.g.*, the Pfam database, release 2.1) using the default parameters ([http://www.sanger.ac.uk/Software/Pfam/HMM\\_search](http://www.sanger.ac.uk/Software/Pfam/HMM_search)). For

example, the hmmsf program, which is available as part of the HMMER package of search programs, is a family specific default program for MILPAT0063 and a score of 15 is the default threshold score for determining a hit. Alternatively, the threshold score for determining a hit can be lowered (*e.g.*, to 8 bits). A description of the Pfam database can be found in Sonhammer *et al.* (1997) *Proteins* 28(3):405-420 and a detailed description of HMMs can be found, for example, in Gribskov *et al.* (1990) *Meth. Enzymol.* 183:146-159; Gribskov *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:4355-4358; Krogh *et al.* (1994) *J. Mol. Biol.* 235:1501-1531; and Stultz *et al.* (1993) *Protein Sci.* 2:305-314, the contents of which are incorporated herein by reference. A search was performed against the HMM database resulting in the identification of an amino acid sequence of human 26583 that is homologous to a sequence contained in PP2C at about residues 172 to 461 of SEQ ID NO:2 (see Figure 3A). The search further identified an amino acid sequence of human 26583 that is homologous to a sequence contained in PP2C\_4 at about residues 99 to 523 of SEQ ID NO:2 (see Figure 3B).

As the 26583 polypeptides of the invention may modulate 26583-mediated activities, they may be useful as of for developing novel diagnostic and therapeutic agents for 26583-mediated or related disorders, as described below. As used herein, "26583 activity," "biological activity of 26583" or "functional activity of 26583," refers to an activity exerted by a 26583 protein, polypeptide or nucleic acid molecule on *e.g.*, a 26583-responsive cell or on a 26583 substrate, *e.g.*, a protein substrate, as determined *in vivo* or *in vitro*. In one embodiment, a 26583 activity is a direct activity, such as an association with a 26583 target molecule. A "target molecule" or "binding partner" is a molecule with which a 26583 protein binds or interacts with in nature, *e.g.*, a protein containing one or more serine/threonine residues. A 26583 activity also can be an indirect activity, *e.g.*, a cellular signaling activity mediated by interaction of the 26583 protein with a 26583 receptor (*e.g.*, a receptor that is a protein serine/threonine kinase). For example, a 26583 protein of the present invention can have one or more of the following activities: (1) removal of phosphate moieties from phospho-serine/threonine residues in proteins; (2) the regulation of transmission of signals from cellular receptors; (3) modulation of cellular growth signaling mechanisms; (4) modulation of cell proliferation; (5) modulation of cell differentiation; (6) modulation of transformation; (7) modulation of apoptosis (*e.g.*, a



cancer cell); (8) modulation of cholesterol biosynthesis; (9) modulation of glycogen metabolism; (10) modulation of muscle contractility; (11) modulation of calcium ion channel activity; (12) modulation of glycolysis, glycogenolysis and gluconeogenesis; (13) modulation of amino-acid degradation; (14) modulation of lipid metabolism;  
 5 and/or (15) modulation of catecholamine synthesis.

As used herein, the term “cellular growth signaling mechanism” includes the ability to interact with, *e.g.*, bind to, and remove a phospho-serine/threonine residue present in a protein, *e.g.*, a serine or threonine phosphorylated protein and modulate, *e.g.*, inhibit, one or more of: (1) induction of receptor dimerization, (2)  
 10 serine/threonine kinase activation, (3) phosphorylation of signaling molecules, and/or (4) induction gene expression; thereby regulating one or more of: (5) cell proliferation, (6) cell differentiation, (7) cell survival, (8) oncogenic transformation, (9) migration, and/or (10) apoptosis, of a cell (*e.g.*, a cancer cell), (11) modulation of cholesterol biosynthesis, (12) modulation of glycogen metabolism, (13) modulation of  
 15 muscle contractility, (14) modulation of calcium ion channel activity, (15) modulation of glycolysis, glycogenolysis and gluconeogenesis, (16) modulation of amino-acid degradation, (17) modulation of lipid metabolism and/or (18) modulation of catecholamine synthesis.

Based on the above-described sequence similarities, a 26583 molecule of the  
 20 present invention is predicted to have similar biological activities as serine/threonine phosphatase family members. Thus, the 26583 molecules can act as novel diagnostic targets and therapeutic agents for controlling cellular proliferative disorders or metabolic disorders such as those associated with cholesterol biosynthesis or mitochondrial dysfunction.

As used herein, a “cholesterol biosynthesis-associated disorder” includes any disorder wherein the regulation of cholesterol biosynthesis is affected by the presence or absence of a 25583 activity of the invention. For example, the 26583 protein of the present invention contains sequence homology to PP2C (see Figure 3A). PP2C possesses high activity against hydroxymethylglutaryl-CoA reductase kinase, which  
 30 inactivates HMG-CoA reductase, the rate-limiting enzyme of the cholesterol biosynthetic pathway. Thus, the present invention provides a means for diagnosing and/or treating a cholesterol biosynthesis-associated disorder such as, for example, hypo- or hypercholesterolemia.

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As previously noted, the 26583 protein of the present invention contains sequence homology to PP2C (see Figure 3A). PP2C appears to be significantly similar to the catalytic subunit of pyruvate dehydrogenase phosphatase (EC 3.1.3.43) (PDPC) that catalyzes dephosphorylation and concomitant reactivation of the alpha subunit of the E1 component of the pyruvate dehydrogenase complex. PDPC is a mitochondrial enzyme and, like PP2C, is magnesium-dependent. Thus, the present invention is additionally useful as a means for diagnosing and/or treating disorders associated with mitochondria. As used herein, a "mitochondrial-associated disorder" includes any disorder related to the function or dysfunction of mitochondria. For example, diabetes mellitus has been associated with deficient mitochondrial oxidative phosphorylation. Also, mitochondrial dysfunction has been implicated in neuro-degenerative disorders, such as Parkinson's, Huntington's and Alzheimer's diseases.

In addition, the 26583 molecules of the invention are useful for diagnosing and/or treating cellular proliferative disorders. As used herein, a "cellular proliferative disorder" includes a disorder, disease, or condition characterized by a deregulated, *e.g.*, up-regulated or down-regulated, growth response. As used herein, a "cellular differentiative disorder" includes a disorder, disease, or condition characterized by aberrant cellular differentiation. As used herein, metastatic refers to the ability of a tumor cell to form implants at a site distant from the original tumor. Thus, the 26583 molecules can act as novel diagnostic targets and therapeutic agents for controlling cellular proliferative and/or differentiative disorders.

Based on the above-described sequence similarities, the 26583 molecules of the present invention are predicted to have similar biological activities as serine/threonine phosphatase family members. Thus, the 26583 molecules can act as novel diagnostic targets and therapeutic agents for controlling one or more of cellular proliferative and/or differentiative disorders, disorders associated with bone metabolism, immune or hematopoietic disorders, cardiovascular disorders, liver disorders, viral diseases, pain or metabolic disorders such as hypo- or hypercholesterolemia, or disorders associated with mitochondrial dysfunction.

In addition, the 26583 molecules of the invention may modulate physiological and pathological processes in the cells or tissues where they are expressed. For example, Taq Man studies described herein show expression of 26583 in normal human breast, lung, colon, liver, and brain tissue (Figure 4). 26583 expression can be

modulated in samples of tumor tissue compared to normal tissue. For example, 26583 expression in brain tumor samples can be significantly higher than in normal brain tissue samples; and 26583 expression in lung tumor tissue can be higher than in normal lung tissue (Figure 4).

5           Examples of cellular proliferative and/or differentiative disorders include cancer, *e.g.*, carcinoma, sarcoma, metastatic disorders or hematopoietic neoplastic disorders, *e.g.*, leukemias. A metastatic tumor can arise from a multitude of primary tumor types, including but not limited to those of prostate, colon, lung, breast and liver origin.

10           As used herein, the terms “cancer,” “hyperproliferative” and “neoplastic” refer to cells having the capacity for autonomous growth, *i.e.*, an abnormal state or condition characterized by rapidly proliferating cell growth. Hyperproliferative and neoplastic disease states may be categorized as pathologic, *i.e.*, characterizing or constituting a disease state, or may be categorized as non-pathologic, *i.e.*, a deviation  
15 from normal but not associated with a disease state. The term is meant to include all types of cancerous growths or oncogenic processes, metastatic tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. “Pathologic hyperproliferative” cells occur in disease states characterized by malignant tumor growth. Examples of non-pathologic  
20 hyperproliferative cells include proliferation of cells associated with wound repair.

          The terms “cancer” or “neoplasms” include malignancies of the various organ systems, such as affecting lung, breast, thyroid, lymphoid, gastrointestinal, and genito-urinary tract, as well as adenocarcinomas which include malignancies such as most colon cancers, renal-cell carcinoma, prostate cancer and/or testicular tumors,  
25 non-small cell carcinoma of the lung, cancer of the small intestine and cancer of the esophagus.

          The term “carcinoma” is art recognized and refers to malignancies of epithelial or endocrine tissues including respiratory system carcinomas, gastrointestinal system carcinomas, genitourinary system carcinomas, testicular carcinomas, breast  
30 carcinomas, prostatic carcinomas, endocrine system carcinomas, and melanomas. Exemplary carcinomas include those forming from tissue of the cervix, lung, prostate, breast, head and neck, colon and ovary. The term also includes carcinosarcomas, *e.g.*, which include malignant tumors composed of carcinomatous and sarcomatous tissues.

The term “sarcoma” is art recognized and refers to malignant tumors of mesenchymal derivation.

10 Preferably, the diseases arise from poorly differentiated acute leukemias, *e.g.*,  
erythroblastic leukemia and acute megakaryoblastic leukemia. Additional exemplary  
myeloid disorders include, but are not limited to, acute promyeloid leukemia (APML),  
acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML)  
(reviewed in Vaickus (1991) *Crit Rev. in Oncol./Hematol.* 11:267-97); lymphoid  
15 malignancies include, but are not limited to acute lymphoblastic leukemia (ALL)  
which includes B-lineage ALL and T-lineage ALL, chronic lymphocytic leukemia  
(CLL), prolymphocytic leukemia (PLL), hairy cell leukemia (HLL) and  
Waldenstrom's macroglobulinemia (WM). Additional forms of malignant  
lymphomas include, but are not limited to non-Hodgkin lymphoma and variants  
20 thereof, peripheral T cell lymphomas, adult T cell leukemia/lymphoma (ATL),  
cutaneous T-cell lymphoma (CTCL), large granular lymphocytic leukemia (LGF),  
Hodgkin's disease and Reed-Sternberg disease.

Aberrant expression and/or activity of 26583 molecules may mediate disorders associated with bone metabolism. “Bone metabolism” refers to direct or indirect effects in the formation or degeneration of bone structures, *e.g.*, bone formation, bone resorption, etc., which may ultimately affect the concentrations in serum of calcium and phosphate. This term also includes activities mediated by 26583 molecules effects in bone cells, *e.g.*, osteoclasts and osteoblasts, that may in turn result in bone formation and degeneration. For example, 26583 molecules may support different activities of bone resorbing osteoclasts, such as the stimulation of differentiation of monocytes and mononuclear phagocytes into osteoclasts. Accordingly, 26583 molecules that modulate the production of bone cells can influence bone formation and degeneration, and thus may be used to treat bone disorders. Examples of such

disorders include, but are not limited to, osteoporosis, osteodystrophy, osteomalacia, rickets, osteitis fibrosa cystica, renal osteodystrophy, osteosclerosis, anti-convulsant treatment, osteopenia, fibrogenesis-imperfecta ossium, secondary hyperparathyroidism, hypoparathyroidism, hyperparathyroidism, cirrhosis, obstructive jaundice, drug induced metabolism, medullary carcinoma, chronic renal disease, rickets, sarcoidosis, glucocorticoid antagonism, malabsorption syndrome, steatorrhea, tropical sprue, idiopathic hypercalcemia and milk fever.

Examples of immune disorders or diseases include, but are not limited to, autoimmune diseases (including, for example, diabetes mellitus, arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis), multiple sclerosis, encephalomyelitis, myasthenia gravis, systemic lupus erythematosus, autoimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), psoriasis, Sjögren's Syndrome, Crohn's disease, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, asthma, allergic asthma, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions, leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis, acute necrotizing hemorrhagic encephalopathy, idiopathic bilateral progressive sensorineural hearing loss, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Stevens-Johnson syndrome, idiopathic sprue, lichen planus, Graves' disease, sarcoidosis, primary biliary cirrhosis, uveitis posterior, and interstitial lung fibrosis), graft-versus-host disease, cases of transplantation, and allergy such as, atopic allergy.

Examples of disorders involving the heart or "cardiovascular disorder" include, but are not limited to, a disease, disorder, or state involving the cardiovascular system, *e.g.*, the heart, the blood vessels, and/or the blood. A cardiovascular disorder can be caused by an imbalance in arterial pressure, a malfunction of the heart, or an occlusion of a blood vessel, *e.g.*, by a thrombus. Examples of such disorders include hypertension, atherosclerosis, coronary artery spasm, congestive heart failure, coronary artery disease, valvular disease, arrhythmias, and cardiomyopathies.

Disorders which may be treated or diagnosed by methods described herein include, but are not limited to, disorders associated with an accumulation in the liver

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of fibrous tissue, such as that resulting from an imbalance between production and degradation of the extracellular matrix accompanied by the collapse and condensation of preexisting fibers. The methods described herein can be used to diagnose or treat hepatocellular necrosis or injury induced by a wide variety of agents including processes which disturb homeostasis, such as an inflammatory process, tissue damage resulting from toxic injury or altered hepatic blood flow, and infections (*e.g.*, bacterial, viral and parasitic). For example, the methods can be used for the early detection of hepatic injury, such as portal hypertension or hepatic fibrosis. In addition, the methods can be employed to detect liver fibrosis attributed to inborn errors of metabolism, for example, fibrosis resulting from a storage disorder such as Gaucher's disease (lipid abnormalities) or a glycogen storage disease, A1-antitrypsin deficiency; a disorder mediating the accumulation (*e.g.*, storage) of an exogenous substance, for example, hemochromatosis (iron-overload syndrome) and copper storage diseases (Wilson's disease), disorders resulting in the accumulation of a toxic metabolite (*e.g.*, tyrosinemia, fructosemia and galactosemia) and peroxisomal disorders (*e.g.*, Zellweger syndrome). Additionally, the methods described herein may be useful for the early detection and treatment of liver injury associated with the administration of various chemicals or drugs, such as, for example, methotrexate, isoniazid, oxyphenisatin, methyldopa, chlorpromazine, tolbutamide or alcohol, or which represents a hepatic manifestation of a vascular disorder such as obstruction of either the intrahepatic or extrahepatic bile flow or an alteration in hepatic circulation resulting, for example, from chronic heart failure, veno-occlusive disease, portal vein thrombosis or Budd-Chiari syndrome.

Additionally, 26583 molecules may play an important role in the etiology of certain viral diseases, including, but not limited to, Hepatitis B, Hepatitis C, and Herpes Simplex Virus (HSV). Modulators of 26583 activity could be used to control viral diseases. The modulators can be used in the treatment and/or diagnosis of viral infected tissue or virus-associated tissue fibrosis, especially liver and liver fibrosis. Also, 26583 modulators can be used in the treatment and/or diagnosis of virus-associated carcinoma, especially hepatocellular cancer.

Additionally, 26583 may play an important role in the regulation of metabolism or pain disorders. Diseases of metabolic imbalance include, but are not limited to, obesity, anorexia nervosa, cachexia, lipid disorders or diabetes. Examples

of pain disorders include, but are not limited to, pain response elicited during various forms of tissue injury, *e.g.*, inflammation, infection, and ischemia, usually referred to as hyperalgesia (described in, for example, Fields, H.L. (1987) *Pain*, New York:McGraw-Hill); pain associated with musculoskeletal disorders, *e.g.*, joint pain; tooth pain; headaches; pain associated with surgery; pain related to irritable bowel syndrome; or chest pain.

The 26583 protein, fragments thereof, and derivatives and other variants of the sequence in SEQ ID NO:2 thereof are collectively referred to as “polypeptides or proteins of the invention” or “26583 polypeptides or proteins.” Nucleic acid molecules encoding such polypeptides or proteins are collectively referred to as “nucleic acids of the invention” or “26583 nucleic acids.” 26583 molecules refer to 26583 nucleic acids, polypeptides, and antibodies.

As used herein, the term “nucleic acid molecule” includes DNA molecules (*e.g.*, a cDNA or genomic DNA) and RNA molecules (*e.g.*, an mRNA) and analogs of the DNA or RNA generated, *e.g.*, by the use of nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

The term “isolated or purified nucleic acid molecule” includes nucleic acid molecules that are separated from other nucleic acid molecules that are present in the natural source of the nucleic acid. For example, with respect to genomic DNA, the term “isolated” includes nucleic acid molecules that are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an “isolated” nucleic acid is free of sequences that naturally flank the nucleic acid (*i.e.*, sequences located at the 5’ and/or 3’ ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of 5’ and/or 3’ nucleotide sequences that naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an “isolated” nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

As used herein, the term “hybridizes under low stringency, medium stringency, high stringency, or very high stringency conditions” describes conditions for hybridization and washing. Guidance for performing hybridization reactions can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y.

5 (1989), 6.3.1-6.3.6, which is incorporated by reference. Aqueous and nonaqueous methods are described in that reference and either can be used. Specific hybridization conditions referred to herein are as follows: 1) low stringency hybridization conditions in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by two washes in 0.2X SSC, 0.1% SDS at least at 50°C (the temperature of the washes  
10 can be increased to 55°C for low stringency conditions); 2) medium stringency hybridization conditions in 6X SSC at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 60°C; 3) high stringency hybridization conditions in 6X SSC at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 65°C; and preferably 4) very high stringency hybridization conditions are 0.5M sodium  
15 phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2X SSC, 1% SDS at 65°C. Very high stringency conditions (4) are the preferred conditions and the ones that should be used unless otherwise specified.

As used herein, a “naturally-occurring” nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*,  
20 encodes a natural protein).

As used herein, the terms “gene” and “recombinant gene” refer to nucleic acid molecules that include an open reading frame encoding a 26583 protein, preferably a mammalian 26583 protein, and further can include non-coding regulatory sequences and introns.

25 An “isolated” or “purified” polypeptide or protein is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. In one embodiment, the language “substantially free” means preparation of 26583 protein having less than about 30%,  
30 20%, 10% and more preferably 5% (by dry weight), of non-26583 protein (also referred to herein as a “contaminating protein”), or of chemical precursors or non-26583 chemicals. When the 26583 protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium,



*i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation. The invention includes isolated or purified preparations of at least 0.01, 0.1, 1.0, and 10 milligrams in dry weight.

5 A “non-essential” amino acid residue is a residue that can be altered from the wild-type sequence of 26583 (*e.g.*, the sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_) without abolishing or more preferably, without substantially altering a biological activity of the 26583 protein, whereas an “essential”  
10 amino acid residue results in such a change. For example, amino acid residues that are conserved among the polypeptides of the present invention, *e.g.*, those present in the phosphatase catalytic domain, are predicted to be particularly unamenable to alteration.

A “conservative amino acid substitution” is one in which the amino acid  
15 residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar  
20 side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a 26583 protein is preferably replaced with another amino acid residue from the same side chain family.  
25 Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a 26583 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for 26583 biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession  
30 Number \_\_\_\_\_, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

As used herein, a “biologically active portion” of a 26583 protein includes a fragment of a 26583 protein that participates in an interaction between a 26583

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molecule and a non-26583 molecule. Biologically active portions of a 26583 protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the 26583 protein, *e.g.*, the amino acid sequence shown in SEQ ID NO:2, which include less amino acids than the full length

5 26583 protein and exhibit at least one activity of a 26583 protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the 26583 protein, *e.g.*, serine/threonine phosphatase activity. A biologically active portion of a 26583 protein can be a polypeptide that is, for example, 10, 25, 50, 100, 200 or more amino acids in length. Biologically active portions of a 26583 protein

10 can be used as targets for developing agents that modulate a 26583 mediated activity, *e.g.*, serine/threonine phosphatase activity.

Particularly preferred 26583 polypeptides of the present invention have an amino acid sequence substantially identical to the amino acid sequence of SEQ ID NO:2. In the context of an amino acid sequence, the term "substantially identical" is

15 used herein to refer to a first amino acid that contains a sufficient or minimum number of amino acid residues that are i) identical to, or ii) conservative substitutions of aligned amino acid residues in a second amino acid sequence such that the first and second amino acid sequences can have a common structural domain and/or common functional activity. For example, amino acid sequences that contain a common

20 structural domain having at least about 60%, or 65% identity, likely 75% identity, more likely 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO:2 are termed substantially identical.

In the context of nucleotide sequence, the term "substantially identical" is used herein to refer to a first nucleic acid sequence that contains a sufficient or minimum

25 number of nucleotides that are identical to aligned nucleotides in a second nucleic acid sequence such that the first and second nucleotide sequences encode a polypeptide having common functional activity, or encode a common structural polypeptide domain or a common functional polypeptide activity. For example, nucleotide sequences having at least about 60%, or 65% identity, likely 75% identity,

30 more likely 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO:1 or 3 are termed substantially identical.

Calculations of homology or sequence identity between sequences (the terms are used interchangeably herein) are performed as follows.

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To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, 90%, 100% of the length of the reference sequence (e.g., when aligning a second sequence to the 26583 amino acid sequence of SEQ ID NO:2 having 537 amino acid residues, at least 162, preferably at least 216, more preferably at least 270, even more preferably at least 324, and even more preferably at least 378, 432 or 486 amino acid residues are aligned). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that should be used if the

practitioner is uncertain about what parameters should be applied to determine if a molecule is within the invention) is using a Blossum 62 scoring matrix with a gap open penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

The percent identity between two amino acid or nucleotide sequences can be determined using the algorithm of Meyers and Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences described herein can be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to 26583 nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to 26583 protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

"Misexpression or aberrant expression", as used herein, refers to a non-wild type pattern of gene expression, at the RNA or protein level. It includes: expression at non-wild type levels, i.e., over or under expression; a pattern of expression that differs from wild type in terms of the time or stage at which the gene is expressed, e.g., increased or decreased expression (as compared with wild type) at a predetermined developmental period or stage; a pattern of expression that differs from wild type in terms of decreased expression (as compared with wild type) in a predetermined cell type or tissue type; a pattern of expression that differs from wild type in terms of the splicing size, amino acid sequence, post-translational modification, or biological activity of the expressed polypeptide; a pattern of expression that differs from wild type in terms of the effect of an environmental stimulus or extracellular stimulus on expression of the gene, e.g., a pattern of increased or decreased

expression (as compared with wild type) in the presence of an increase or decrease in the strength of the stimulus.

"Subject," as used herein, refers to human and non-human animals. The term "non-human animals" of the invention includes all vertebrates, e.g., mammals, such as non-human primates (particularly higher primates), sheep, dog, rodent (e.g., mouse or rat), guinea pig, goat, pig, cat, rabbits, cow, and non-mammals, such as chickens, amphibians, reptiles, etc. In a preferred embodiment, the subject is a human. In another embodiment, the subject is an experimental animal or animal suitable as a disease model.

A "purified preparation of cells", as used herein, refers to, in the case of plant or animal cells, an in vitro preparation of cells and not an entire intact plant or animal. In the case of cultured cells or microbial cells, it consists of a preparation of at least 10% and more preferably 50% of the subject cells.

Various aspects of the invention are described in further detail below.

#### Isolated Nucleic Acid Molecules

In one aspect, the invention provides an isolated or purified nucleic acid molecule that encodes a 26583 polypeptide described herein, e.g., a full-length 26583 protein or a fragment thereof, e.g., a biologically active portion of a 26583 protein.

Also included is a nucleic acid fragment suitable for use as a hybridization probe, which can be used, e.g., to identify a nucleic acid molecule encoding a polypeptide of the invention, 26583 mRNA, or fragments suitable for use as primers, e.g., PCR primers for the amplification or mutation of nucleic acid molecules.

In one embodiment, an isolated nucleic acid molecule of the invention includes the nucleotide sequence shown in SEQ ID NO:1, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_, or a portion of any of these nucleotide sequences. In one embodiment, the nucleic acid molecule includes sequences encoding the 26583 protein (*i.e.*, "the coding region," from nucleotides 462-2075 of SEQ ID NO:1), as well as 5' untranslated sequences (nucleotides 1-461 of SEQ ID NO:1). Alternatively, the nucleic acid molecule can include only the coding region of SEQ ID NO:1 (*e.g.*, nucleotides 462-2075, corresponding to SEQ ID NO:3) and, *e.g.*, no flanking sequences that normally accompany the subject sequence. In another embodiment, the nucleic acid molecule

encodes a sequence corresponding to the mature protein from about amino acid 1 to amino acid 537 of SEQ ID NO:2.

In another embodiment, an isolated nucleic acid molecule of the invention includes a nucleic acid molecule that is a complement of the nucleotide sequence shown in SEQ ID NO:1 or 3, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_, or a portion of any of these nucleotide sequences. In other embodiments, the nucleic acid molecule of the invention is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_ such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_, thereby forming a stable duplex.

In one embodiment, an isolated nucleic acid molecule of the present invention includes a nucleotide sequence that is at least about: 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more homologous to the entire length of the nucleotide sequence shown in SEQ ID NO:1 or 3, or the entire length of the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_. In the case of an isolated nucleic acid molecule which is longer than or equivalent in length to the reference sequence, *e.g.*, SEQ ID NO:1, the comparison is made with the full length of the reference sequence. Where the isolated nucleic acid molecule is shorter than the reference sequence, *e.g.*, shorter than SEQ ID NO:1, the comparison is made to a segment of the reference sequence of the same length (excluding any loop required by the homology calculation).

#### 26583 Nucleic Acid Fragments

A nucleic acid molecule of the invention can include only a portion of the nucleic acid sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_. For example, such a nucleic acid molecule can include a fragment that can be used as a probe or primer or a fragment encoding a portion of a 26583 protein, *e.g.*, an immunogenic or biologically active portion of a 26583 protein. A fragment can

comprise a nucleotides encoding amino acids 172 to 461 or 99 to 523 of SEQ ID NO:2, which encode a phosphatase catalytic domain of human 26583. The nucleotide sequence determined from the cloning of the 26583 gene allows for the generation of probes and primers designed for use in identifying and/or cloning other 26583 family members, or fragments thereof, as well as 26583 homologues or fragments thereof, from other species.

In another embodiment, a nucleic acid includes a nucleotide sequence that includes part, or all, of the coding region and extends into either (or both) the 5' or 3' noncoding region. Other embodiments include a fragment that includes a nucleotide sequence encoding an amino acid fragment described herein. Nucleic acid fragments can encode a specific domain or site described herein or fragments thereof, particularly fragments thereof which are at least 400 amino acids in length. Fragments also include nucleic acid sequences corresponding to specific amino acid sequences described above or fragments thereof. Nucleic acid fragments should not to be construed as encompassing those fragments that may have been disclosed prior to the invention.

A nucleic acid fragment can include a sequence corresponding to a domain, region, or functional site described herein. A nucleic acid fragment also can include one or more domains, regions, or functional sites described herein. Thus, for example, the nucleic acid fragment can include a serine/threonine phosphatase catalytic domain, a protein kinase C phosphorylation site, an N-glycosylation site, a casein kinase II phosphorylation site, an N-myristoylation site, an amidation site, a protein phosphatase 2C signature domain, or any combination thereof.

In a preferred embodiment, the fragment is at least 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, or 1000 nucleotides in length, and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1, or SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_.

26583 probes and primers are provided. Typically a probe/primer is an isolated or purified oligonucleotide. The oligonucleotide typically includes a region of nucleotide sequence that hybridizes under stringent conditions to at least about 7, 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 consecutive nucleotides of a sense or antisense sequence of SEQ ID NO:1 or 3, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as

Accession Number \_\_\_\_\_, of a naturally occurring allelic variant or mutant of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_.

In a preferred embodiment the nucleic acid is a probe that is at least 5 or 10  
 5 and less than 500, 300, or 200 base pairs in length, and more preferably is less than 100 or less than 50 base pairs in length. It should be identical, or differ by 1, or less than 5 or 10 bases, from a sequence disclosed herein. If alignment is needed for this comparison, the sequences should be aligned for maximum homology. "Looped" out sequences in the alignment from deletions, insertions, or mismatches, are considered  
 10 differences.

A probe or primer can be derived from the sense or anti-sense strand of a nucleic acid that encodes a serine/threonine phosphatase catalytic domain: amino acids 172 to 461 or 99 to 523 of SEQ ID NO:2.

In another embodiment a set of primers is provided, *e.g.*, primers suitable for  
 15 use in a PCR, which can be used to amplify a selected region of a 26583 sequence, *e.g.*, a region, domain, or site described herein. The primers should be at least 5, 10, or 50 base pairs in length and less than 100 or 200 base pairs in length. The primers should be identical, or differ by one base from a sequence disclosed herein or from a naturally occurring variant. *E.g.*, primers suitable for amplifying all or a portion of  
 20 the serine/threonine phosphatase catalytic domain (amino acid residues 172 to 461 or 99 to 523 of SEQ ID NO:2).

A nucleic acid fragment can encode an epitope bearing region of a polypeptide described herein.

A nucleic acid fragment encoding a "biologically active portion of a 26583  
 25 polypeptide" can be prepared by isolating a portion of the nucleotide sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_, which encodes a polypeptide having a 26583 biological activity (*e.g.*, the biological activities of the 26583 proteins described herein), expressing the encoded portion of the 26583 protein (*e.g.*, by  
 30 recombinant expression *in vitro*) and assessing the activity of the encoded portion of the 26583 protein. For example, a nucleic acid fragment encoding a biologically active portion of 26583 includes a serine/threonine phosphatase catalytic domain, *e.g.*, amino acid residues 99 to 523 of SEQ ID NO:2. A nucleic acid fragment encoding a



biologically active portion of a 26583 polypeptide, may comprise a nucleotide sequence that is greater than about 300 or more nucleotides in length (*e.g.*, greater than about 400 nucleotides in length).

In preferred embodiments, a nucleic acid includes a nucleotide sequence which is about 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300 or more nucleotides in length and hybridizes under a stringency condition described herein to a nucleic acid molecule of SEQ ID NO:1, or SEQ ID NO:3.

#### 26583 Nucleic Acid Variants

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_. Such differences can be due to degeneracy of the genetic code (and result in a nucleic acid that encodes the same 26583 proteins as those encoded by the nucleotide sequence disclosed herein. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence that differs by at least 1, but less than 5, 10, 20, 50, or 100 amino acid residues than that shown in SEQ ID NO:2. If alignment is needed for this comparison the sequences should be aligned for maximum homology. "Looped" out sequences from deletions, insertions, or mismatches, are considered differences.

Nucleic acids of the invention can be chosen for having codons, which are preferred, or non-preferred, for a particular expression system (*e.g.*, the nucleic acid can be one in which at least one codon, at preferably at least 10%, or 20% of the codons has been altered such that the sequence is optimized for expression in *E. coli*, yeast, human, insect, or chinese hamster ovary (CHO) cells).

Nucleic acid variants can be naturally occurring, such as allelic variants (same locus), homologs (different locus), and orthologs (different organism) or can be non-naturally occurring. Non-naturally occurring variants can be made by mutagenesis techniques, including those applied to polynucleotides, cells, or organisms. The variants can contain nucleotide substitutions, deletions, inversions, and insertions. Variation can occur in either or both the coding and non-coding regions. The variations can produce both conservative and non-conservative amino acid substitutions (as compared with the encoded product).

In a preferred embodiment, the nucleic acid differs from that of SEQ ID NO: 1 or 3, or the sequence in ATCC Accession Number \_\_\_, *e.g.*, as follows: by at least one but less than 10, 20, 30, or 40 nucleotides; at least one but less than 1%, 5%, 10% or 20% of the nucleotides in the subject nucleic acid. If necessary for this analysis, the sequences should be aligned for maximum homology. "Looped" out sequences from deletions, insertions, or mismatches, are considered differences.

Orthologs, homologs, and allelic variants can be identified using methods known in the art. These variants comprise a nucleotide sequence encoding a polypeptide that is 50%, at least about 55%, typically at least about 70-75%, more typically at least about 80-85%, and most typically at least about 90-95% or more identical to the amino acid sequence shown in SEQ ID NO:2 or a fragment of this sequence. Such nucleic acid molecules can be obtained as being able to hybridize under stringent conditions, to the nucleotide sequence shown in SEQ ID NO 1 or a fragment of the sequence. Nucleic acid molecules corresponding to orthologs, homologs, and allelic variants of the 26583 cDNAs of the invention can further be isolated by mapping to the same chromosome or locus as the 26583 gene. Preferred variants include those that are correlated with serine/threonine phosphatase activity.

Orthologs, homologs, and allelic variants can be identified using methods known in the art. These variants comprise a nucleotide sequence encoding a polypeptide that is 50%, at least about 55%, typically at least about 70-75%, more typically at least about 80-85%, and most typically at least about 90-95% or more identical to the amino acid sequence shown in SEQ ID NO:2 or a fragment of this sequence. Such nucleic acid molecules can readily be identified as being able to hybridize under stringent conditions, to the nucleotide sequence shown in SEQ ID NO 1 or a fragment of the sequence. Nucleic acid molecules corresponding to orthologs, homologs, and allelic variants of the 26583 cDNAs of the invention can further be isolated by mapping to the same chromosome or locus as the 26583 gene. Preferred variants include those that are correlated with phosphatase activity, *e.g.*, serine/threonine phosphatase activity.

Allelic variants of 26583, *e.g.*, human 26583, include both functional and non-functional proteins. Functional allelic variants are naturally occurring amino acid sequence variants of the 26583 protein within a population that maintain the ability to remove the phosphate from a serine or threonine residue of a phosphorylated protein. Functional allelic variants typically will contain only conservative substitution of one

or more amino acids of SEQ ID NO:2, or substitution, deletion or insertion of non-critical residues in non-critical regions of the protein. Non-functional allelic variants are naturally-occurring amino acid sequence variants of the 26583, *e.g.*, human 26583, protein within a population that do not have the ability to remove the phosphate from a serine or threonine residue of a phosphorylated protein. Non-functional allelic variants will typically contain a non-conservative substitution, a deletion, or insertion, or premature truncation of the amino acid sequence of SEQ ID NO:2, or a substitution, insertion, or deletion in critical residues or critical regions of the protein.

Moreover, nucleic acid molecules encoding other 26583 family members and, thus have a nucleotide sequence that differs from the 26583 sequences of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_ are intended to be within the scope of the invention.

#### Antisense Nucleic Acid Molecules, Ribozymes and Modified 26583 Nucleic Acid Molecules

In another aspect, the invention features, an isolated nucleic acid molecule that is antisense to 26583. An “antisense” nucleic acid can include a nucleotide sequence that is complementary to a “sense” nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. The antisense nucleic acid can be complementary to an entire 26583 coding strand, or to only a portion thereof (*e.g.*, the coding region of 26583 corresponding to SEQ ID NO:3). In another embodiment, the antisense nucleic acid molecule is antisense to a “noncoding region” of the coding strand of a nucleotide sequence encoding 26583 (*e.g.*, the 5' and 3' untranslated regions).

An antisense nucleic acid can be designed such that it is complementary to the entire coding region of 26583 mRNA, but more preferably is an oligonucleotide that is antisense to only a portion of the coding or noncoding region of 26583 mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of 26583 mRNA, *e.g.*, between the -10 and +10 regions of the target gene nucleotide sequence. An antisense oligonucleotide can be,

for example, about 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, or more nucleotides in length.

An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions with procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used. The antisense nucleic acid also can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject (*e.g.*, by direct injection at a tissue site), or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a 26583 protein to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies that bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong polymerase II or polymerase III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an  $\alpha$ -anomeric nucleic acid molecule. An  $\alpha$ -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual  $\beta$ -units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids. Res.* 15:6625-6641). The antisense nucleic acid molecule can

also comprise a 2'-o-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a  
 5 ribozyme. A ribozyme having specificity for a 26583-encoding nucleic acid can include one or more sequences complementary to the nucleotide sequence of a 26583 cDNA disclosed herein (*i.e.*, SEQ ID NO:1 or 3), and a sequence having known catalytic sequence responsible for mRNA cleavage (see U.S. Pat. No. 5,093,246 or Haselhoff and Gerlach (1988) *Nature* 334:585-591). For example, a derivative of a  
 10 *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a 26583-encoding mRNA. See, *e.g.*, Cech *et al.* U.S. Patent No. 4,987,071; and Cech *et al.* U.S. Patent No. 5,116,742. Alternatively, 26583 mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules.  
 15 See, *e.g.*, Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

26583 gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the 26583 (*e.g.*, the 26583 promoter and/or enhancers) to form triple helical structures that prevent transcription of the 26583 gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-  
 20 84; Helene, C. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15. The potential sequences that can be targeted for triple helix formation can be increased by creating a "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the  
 25 necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

The invention also provides detectably labeled oligonucleotide primer and probe molecules. Typically, such labels are chemiluminescent, fluorescent, radioactive, or colorimetric.

30 A 26583 nucleic acid molecule can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup

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B. *et al.* (1996) *Bioorganic & Medicinal Chemistry* 4 (1): 5-23). As used herein, the terms "peptide nucleic acid" or "PNA" refers to a nucleic acid mimic, *e.g.*, a DNA mimic in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of a PNA can allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *et al.* *Proc. Natl. Acad. Sci.* 93: 14670-675.

PNAs of 26583 nucleic acid molecules can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of 26583 nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (*e.g.*, by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (*e.g.*, S1 nucleases (Hyrup B. (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *supra*).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (see, *e.g.*, Krol *et al.* (1988) *Bio-Techniques* 6:958-976) or intercalating agents (see, *e.g.*, Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (*e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

The invention also includes molecular beacon oligonucleotide primer and probe molecules having at least one region that is complementary to a 26583 nucleic acid of the invention. The molecular beacon primer and probe molecules also have two complementary regions, one having a fluorophore and one having a quencher, such that the molecular beacon is useful for quantitating the presence of a 26583

nucleic acid of the invention in a sample. Molecular beacon nucleic acids are described, for example, in Lizardi *et al.*, U.S. Patent No. 5,854,033; Nazarenko *et al.*, U.S. Patent No. 5,866,336, and Livak *et al.*, U.S. Patent 5,876,930.

#### 5 Isolated 26583 Polypeptides

In another aspect, the invention features an isolated 26583 protein or fragment thereof, *e.g.*, a biologically active portion for use as immunogens or antigens to raise or test (or more generally to bind) anti-26583 antibodies. 26583 protein can be isolated from cells or tissue sources using standard protein purification techniques.

10 26583 protein or fragments thereof can be produced by recombinant DNA techniques or synthesized chemically.

Polypeptides of the invention include those that arise as a result of the existence of multiple genes, alternative transcription events, alternative RNA splicing events, and alternative translational and postranslational events. The polypeptide can be expressed in systems, *e.g.*, cultured cells, which result in substantially the same postranslational modifications present when expressed the polypeptide is expressed in a native cell, or in systems which result in the alteration or omission of postranslational modifications, *e.g.*, glycosylation or cleavage, present when expressed in a native cell.

20 In a preferred embodiment, a 26583 polypeptide has one or more of the following characteristics:

- (i) it has the ability to promote removal of phosphate from phosphorylated serine or threonine residues of protein;
- (ii) it has a molecular weight (*e.g.*, a deduced molecular weight), amino acid composition or other physical characteristic of a 26583 protein, *e.g.*, a 26583 protein of SEQ ID NO:2;
- (iii) it has an overall sequence similarity of at least 60%, more preferably at least 70, 80, 90, 95%, most preferably at least 99%, with a polypeptide encoded by SEQ ID NO:3;
- 30 (iv) it has a phosphatase catalytic domain which is preferably about 70%, 80%, 90%, 95%, most preferably at least 99%, identical to amino acid residues 99-523 of SEQ ID NO:2;

- (v) it has a phosphatase catalytic domain which is preferably about 70%, 80%, 90%, 95%, most preferably at least 99%, identical to with amino acid residues 172 to 461 of SEQ ID NO:2; or
- (vi) it has at least 70%, preferably at least 80%, and most preferably at least 95% of the cysteines found in the amino acid sequence of the native protein.

In a preferred embodiment, the 26583 protein or fragment thereof differs from the corresponding sequence in SEQ ID NO:2. In one embodiment, it differs by at least one but by less than 15, 10 or 5 amino acid residues. In another embodiment, it differs from the corresponding sequence in SEQ ID NO:2 by at least one residue but less than 20%, 15%, 10% or 5% of the residues in it differ from the corresponding sequence in SEQ ID NO:2. (If this comparison requires alignment, the sequences should be aligned for maximum homology. "Looped" out sequences from deletions, insertions, or mismatches, are considered differences.) The differences are, preferably, differences or changes at a non-essential residue or a conservative substitution. In a preferred embodiment, the differences are not in the serine/threonine phosphatase catalytic domain. In another preferred embodiment one or more differences are at non-active site residues, e.g., amino acids 1-98, or 524 to 537 of SEQ ID NO:2.

Other embodiments include a protein that contains one or more changes in amino acid sequence, e.g., a change in an amino acid residue that is not essential for activity. Such 26583 proteins differ in amino acid sequence from SEQ ID NO:2, yet retain biological activity.

In one embodiment, the protein includes an amino acid sequence at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more homologous to SEQ ID NO:2.

In another embodiment, the protein includes an amino acid sequence at least 213 amino acids in length, and about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, homologous to SEQ ID NO:2.

In another embodiment, a 26583 protein or fragment has an amino acid sequence which differs from the sequence of AAA30697 by at least one, two, three, five or more amino acids. The variations may include the addition, replacement, and/or deletion of amino acid residues.

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109050 250001



A 26583 protein or fragment is provided which varies from the sequence of SEQ ID NO:2 in non-active site residues by at least one but by less than 15, 10 or 5 amino acid residues in the protein or fragment, but which does not differ from SEQ ID NO:2 in regions having phosphatase catalytic activity. (If this comparison requires alignment the sequences should be aligned for maximum homology. "Looped" out sequences from deletions, insertions, or mismatches, are considered differences.) In some embodiments, the difference is at a non-essential residue or is a conservative substitution, while in others, the difference is at an essential residue or is a non conservative substitution.

In one embodiment, a biologically active portion of a 26583 protein includes a serine/threonine phosphatase catalytic domain. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native 26583 protein.

In a preferred embodiment, the 26583 protein has an amino acid sequence shown in SEQ ID NO:2. In other embodiments, the 26583 protein is substantially identical to SEQ ID NO:2. In yet another embodiment, the 26583 protein is substantially identical to SEQ ID NO:2 and retains the functional activity of the protein of SEQ ID NO:2, as described in detail in subsection I above. Accordingly, in another embodiment, the 26583 protein is a protein which includes an amino acid sequence at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more identical to SEQ ID NO:2.

#### 26583 Chimeric or Fusion Proteins

In another aspect, the invention provides 26583 chimeric or fusion proteins. As used herein, a 26583 "chimeric protein" or "fusion protein" includes a 26583 polypeptide linked to a non-26583 polypeptide. A "non-26583 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein that is not substantially homologous to the 26583 protein, *e.g.*, a protein that is different from the 26583 protein and that is derived from the same or a different organism. The 26583 polypeptide of the fusion protein can correspond to all or a portion *e.g.*, a fragment described herein of a 26583 amino acid sequence. In a preferred embodiment, a 26583 fusion protein includes at least one (*e.g.*, two) biologically active portion of a

26583 protein. The non-26583 polypeptide can be fused to the N-terminus or C-terminus of a 26583 polypeptide.

The fusion protein can include a moiety that has high affinity for a ligand. For example, the fusion protein can be a GST-26583 fusion protein in which the 26583 sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant 26583. Alternatively, the fusion protein can be a 26583 protein containing a heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of 26583 can be increased through use of a heterologous signal sequence.

Fusion proteins can include all or a part of a serum protein, *e.g.*, an IgG constant region, or human serum albumin.

The 26583 fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The 26583 fusion proteins can be used to affect the bioavailability of a 26583 substrate. 26583 fusion proteins may be useful therapeutically for the treatment of disorders caused by, for example: (i) aberrant modification or mutation of a gene encoding a 26583 protein; (ii) misregulation of the 26583 gene; and (iii) aberrant post-translational modification of a 26583 protein.

Moreover, 26583-fusion proteins of the invention can be used as immunogens to produce anti-26583 antibodies in a subject, to purify 26583 ligands, and in screening assays to identify molecules that inhibit the interaction of 26583 with a 26583 substrate.

Expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). A 26583-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the 26583 protein.

#### Variants of 26583 Proteins

In another aspect, the invention features a variant of a 26583 polypeptide, *e.g.*, a polypeptide that functions as an agonist (mimetic) or as an antagonist of 26583 activities. Variants of the 26583 proteins can be generated by mutagenesis, *e.g.*, discrete point mutations, the insertion or deletion of sequences or the truncation of a 26583 protein. An agonist of the 26583 protein retains substantially the same, or a

subset, of the biological activities of the naturally occurring form of a 26583 protein.

An antagonist of a 26583 protein can inhibit one or more of the activities of the naturally occurring form of the 26583 protein by, for example, competitively modulating a 26583-mediated activity of a 26583 protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. Preferably, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the 26583 protein.

Variants of a 26583 protein can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of a 26583 protein for agonist or antagonist activity.

Libraries of fragments *e.g.*, N terminal, C terminal, or internal fragments, of a 26583 protein coding sequence can be used to generate a variegated population of fragments for screening and subsequent selection of variants of a 26583 protein.

Variants in which a cysteine residue is added or deleted or in which a residue that is glycosylated is added or deleted are particularly preferred.

Methods for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property are known. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with screening assays to identify 26583 variants (Arkin and Yourvan (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6(3):327-331).

Cell based assays can be exploited to analyze a variegated 26583 library. For example, a library of expression vectors can be transfected into a cell line, *e.g.*, a cell line, which ordinarily responds to 26583 in a substrate-dependent manner. The transfected cells are then contacted with 26583 and the effect of the expression of the mutant on signaling by a 26583 substrate can be detected, *e.g.*, by measuring phosphorylation of serine or threonine residues. Plasmid DNA can then be recovered from the cells that score for inhibition, or alternatively, potentiation of signaling by the 26583 substrate, and the individual clones further characterized.

In another aspect, the invention features a method of making a 26583 polypeptide, *e.g.*, a peptide having a non-wild type activity, *e.g.*, an antagonist,

agonist, or super agonist of a naturally occurring 26583 polypeptide, *e.g.*, a naturally occurring 26583 polypeptide. The method includes: altering the sequence of a 26583 polypeptide, *e.g.*, by substitution or deletion of one or more residues of a non-conserved region, a domain, or residue disclosed herein, and testing the altered polypeptide for the desired activity.

In another aspect, the invention features a method of making a fragment or analog of a 26583 polypeptide that retains at least one biological activity of a naturally occurring 26583 polypeptide. The method includes: altering the sequence, *e.g.*, by substitution or deletion of one or more residues, of a 26583 polypeptide, *e.g.*, altering the sequence of a non-conserved region, or a domain or residue described herein, and testing the altered polypeptide for the desired activity.

#### Anti-26583 Antibodies

In another aspect, the invention provides an anti-26583 antibody, or a fragment thereof (*e.g.*, an antigen-binding fragment thereof). The term "antibody" as used herein refers to an immunoglobulin molecule or immunologically active portion thereof, *i.e.*, an antigen-binding portion. As used herein, the term "antibody" refers to a protein comprising at least one, and preferably two, heavy (H) chain variable regions (abbreviated herein as VH), and at least one and preferably two light (L) chain variable regions (abbreviated herein as VL). The VH and VL regions can be further subdivided into regions of hypervariability, termed "complementarity determining regions" ("CDR"), interspersed with regions that are more conserved, termed "framework regions" (FR). The extent of the framework region and CDR's has been precisely defined (see, Kabat *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia *et al.* (1987) *J. Mol. Biol.* 196:901-917, which are incorporated herein by reference). Each VH and VL is composed of three CDR's and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

The anti-26583 antibody can further include a heavy and light chain constant region, to thereby form a heavy and light immunoglobulin chain, respectively. In one embodiment, the antibody is a tetramer of two heavy immunoglobulin chains and two light immunoglobulin chains, wherein the heavy and light immunoglobulin chains are

inter-connected by, e.g., disulfide bonds. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. The light chain constant region is comprised of one domain, CL. The variable region of the heavy and light chains contains a binding domain that interacts with an antigen. The constant regions of the antibodies typically mediate the binding of the antibody to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system.

As used herein, the term "immunoglobulin" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. The recognized human immunoglobulin genes include the kappa, lambda, alpha (IgA1 and IgA2), gamma (IgG1, IgG2, IgG3, IgG4), delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Full-length immunoglobulin "light chains" (about 25 Kd or 214 amino acids) are encoded by a variable region gene at the NH<sub>2</sub>-terminus (about 110 amino acids) and a kappa or lambda constant region gene at the COOH--terminus. Full-length immunoglobulin "heavy chains" (about 50 Kd or 446 amino acids), are similarly encoded by a variable region gene (about 116 amino acids) and one of the other aforementioned constant region genes, e.g., gamma (encoding about 330 amino acids).

The term "antigen-binding fragment" of an antibody (or simply "antibody portion," or "fragment"), as used herein, refers to one or more fragments of a full-length antibody that retain the ability to specifically bind to the antigen, e.g., 26583 polypeptide or fragment thereof. Examples of antigen-binding fragments of the anti-26583 antibody include, but are not limited to: (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')<sub>2</sub> fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.*,

The anti-26583 antibody can be a polyclonal or a monoclonal antibody. In other embodiments, the antibody can be recombinantly produced, e.g., produced by phage display or by combinatorial methods.

In one embodiment, the anti-26583 antibody is a fully human antibody (e.g., an antibody made in a mouse which has been genetically engineered to produce an antibody from a human immunoglobulin sequence), or a non-human antibody, e.g., a rodent (mouse or rat), goat, primate (e.g., monkey), camel antibody. Preferably, the non-human antibody is a rodent (mouse or rat antibody). Method of producing rodent antibodies are known in the art.

Human monoclonal antibodies can be generated using transgenic mice carrying the human immunoglobulin genes rather than the mouse system. Splenocytes from these transgenic mice immunized with the antigen of interest are used to produce hybridomas that secrete human mAbs with specific affinities for

- epitopes from a human protein (see, e.g., Wood et al. International Application WO 91/00906, Kucherlapati et al. PCT publication WO 91/10741; Lonberg et al. International Application WO 92/03918; Kay et al. International Application 92/03917; Lonberg, N. et al. 1994 *Nature* 368:856-859; Green, L.L. et al. 1994 *Nature Genet.* 7:13-21; Morrison, S.L. et al. 1994 *Proc. Natl. Acad. Sci. USA* 81:6851-6855; Bruggeman et al. 1993 *Year Immunol* 7:33-40; Tuailon et al. 1993 *PNAS* 90:3720-3724; Bruggeman et al. 1991 *Eur J Immunol* 21:1323-1326).

- An anti-26583 antibody can be one in which the variable region, or a portion thereof, e.g., the CDR's, are generated in a non-human organism, e.g., a rat or mouse. Chimeric, CDR-grafted, and humanized antibodies are within the invention. Antibodies generated in a non-human organism, e.g., a rat or mouse, and then modified, e.g., in the variable framework or constant region, to decrease antigenicity in a human are within the invention.

- Chimeric antibodies can be produced by recombinant DNA techniques known in the art. For example, a gene encoding the Fc constant region of a murine (or other species) monoclonal antibody molecule is digested with restriction enzymes to remove the region encoding the murine Fc, and the equivalent portion of a gene encoding a human Fc constant region is substituted (see Robinson et al., International Patent Publication PCT/US86/02269; Akira, et al., European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison et al., European Patent Application 173,494; Neuberger et al., International Application WO 86/01533; Cabilly et al. U.S. Patent No. 4,816,567; Cabilly et al., European Patent Application 125,023; Better et al. (1988 *Science* 240:1041-1043); Liu et al. (1987) *PNAS* 84:3439-3443; Liu et al., 1987, *J. Immunol.* 139:3521-3526; Sun et al. (1987) *PNAS* 84:214-218; Nishimura et al., 1987, *Canc. Res.* 47:999-1005; Wood et al. (1985) *Nature* 314:446-449; and Shaw et al., 1988, *J. Natl Cancer Inst.* 80:1553-1559).

- A humanized or CDR-grafted antibody will have at least one or two but generally all three recipient CDR's (of heavy and or light immunoglobulin chains) replaced with a donor CDR. The antibody may be replaced with at least a portion of a non-human CDR or only some of the CDR's may be replaced with non-human CDR's. It is only necessary to replace the number of CDR's required for binding of the humanized antibody to a 26583 or a fragment thereof. Preferably, the donor will be a rodent

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antibody, e.g., a rat or mouse antibody, and the recipient will be a human framework or a human consensus framework. Typically, the immunoglobulin providing the CDR's is called the "donor" and the immunoglobulin providing the framework is called the "acceptor." In one embodiment, the donor immunoglobulin is a non-human (e.g., rodent). The acceptor framework is a naturally-occurring (e.g., a human) framework or a consensus framework, or a sequence about 85% or higher, preferably 90%, 95%, 99% or higher identical thereto.

As used herein, the term "consensus sequence" refers to the sequence formed from the most frequently occurring amino acids (or nucleotides) in a family of related sequences (See e.g., Winnaker, *From Genes to Clones* (Verlagsgesellschaft, Weinheim, Germany 1987). In a family of proteins, each position in the consensus sequence is occupied by the amino acid occurring most frequently at that position in the family. If two amino acids occur equally frequently, either can be included in the consensus sequence. A "consensus framework" refers to the framework region in the consensus immunoglobulin sequence.

An antibody can be humanized by methods known in the art. Humanized antibodies can be generated by replacing sequences of the Fv variable region which are not directly involved in antigen binding with equivalent sequences from human Fv variable regions. General methods for generating humanized antibodies are provided by Morrison, S. L., 1985, *Science* 229:1202-1207, by Oi et al., 1986, *BioTechniques* 4:214, and by Queen et al. US 5,585,089, US 5,693,761 and US 5,693,762, the contents of all of which are hereby incorporated by reference. Those methods include isolating, manipulating, and expressing the nucleic acid sequences that encode all or part of immunoglobulin Fv variable regions from at least one of a heavy or light chain. Sources of such nucleic acid are well known to those skilled in the art and, for example, may be obtained from a hybridoma producing an antibody against a 26S83 polypeptide or fragment thereof. The recombinant DNA encoding the humanized antibody, or fragment thereof, can then be cloned into an appropriate expression vector.

Humanized or CDR-grafted antibodies can be produced by CDR-grafting or CDR substitution, wherein one, two, or all CDR's of an immunoglobulin chain can be replaced. See e.g., U.S. Patent 5,225,539; Jones et al. 1986 *Nature* 321:552-525; Verhoeyan et al. 1988 *Science* 239:1534; Beidler et al. 1988 *J. Immunol.* 141:4053-

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4060; Winter US 5,225,539, the contents of all of which are hereby expressly incorporated by reference. Winter describes a CDR-grafting method which may be used to prepare the humanized antibodies of the present invention (UK Patent Application GB 2188638A, filed on March 26, 1987; Winter US 5,225,539), the contents of which is expressly incorporated by reference.

Also within the scope of the invention are humanized antibodies in which specific amino acids have been substituted, deleted or added. Preferred humanized antibodies have amino acid substitutions in the framework region, such as to improve binding to the antigen. For example, a humanized antibody will have framework residues identical to the donor framework residue or to another amino acid other than the recipient framework residue. To generate such antibodies, a selected, small number of acceptor framework residues of the humanized immunoglobulin chain can be replaced by the corresponding donor amino acids. Preferred locations of the substitutions include amino acid residues adjacent to the CDR, or which are capable of interacting with a CDR (see e.g., U.S. Patent No. 5,585,089). Criteria for selecting amino acids from the donor are described in US 5,585,089, e.g., columns 12-16 of U.S. Patent No. 5,585,089, the e.g., columns 12-16 of U.S. Patent No. 5,585,089, the contents of which are hereby incorporated by reference. Other techniques for humanizing antibodies are described in Padlan et al. EP 519596 A1, published on December 23, 1992.

In preferred embodiments an antibody can be made by immunizing with purified 26583 antigen, or a fragment thereof, e.g., a fragment described herein; tissue, e.g., crude tissue preparations, whole cells, preferably living cells, lysed cells, or cell fractions, e.g., membrane fractions.

A full-length 26583 protein or, antigenic peptide fragment of 26583 can be used as an immunogen or can be used to identify anti-26583 antibodies made with other immunogens, e.g., cells, membrane preparations, and the like. The antigenic peptide of 26583 should include at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2 and encompass an epitope of 26583. Preferably, the antigenic peptide includes at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

Fragments of 26583 which include residues about 60-70 can be used to make, e.g., used as immunogens or used to characterize the specificity of an antibody, antibodies against hydrophilic regions of the 26583 protein. Similarly, fragments of 26583 which include residues 262-279 can be used to make an antibody against a hydrophobic region of the 26583 protein; a fragment of 26583 which includes residues about 172 to 461 or 99 to 523 can be used to make an antibody against the phosphatase region of the 26583 protein.

Antibodies reactive with, or specific for, any of these regions, or other regions or domains described herein are provided.

Antibodies which bind only native 26583 protein, only denatured or otherwise non-native 26583 protein, or which bind both, are within the invention. Antibodies with linear or conformational epitopes are within the invention. Conformational epitopes can sometimes be identified by identifying antibodies which bind to native but not denatured 26583 protein.

Preferred epitopes encompassed by the antigenic peptide are regions of 26583 are located on the surface of the protein, e.g., hydrophilic regions, as well as regions with high antigenicity. For example, an Emini surface probability analysis of the human 26583 protein sequence can be used to indicate the regions that have a particularly high probability of being localized to the surface of the 26583 protein and are thus likely to constitute surface residues useful for targeting antibody production.

In preferred embodiments antibodies can bind one or more of purified antigen; tissue, e.g., tissue sections; whole cells, preferably living cells; lysed cells; cell fractions.

The anti-26583 antibody can be a single chain antibody. A single-chain antibody (scFV) may be engineered (see, for example, Colcher *et al.* (1999) *Ann N Y Acad Sci* 880:263-80; and Reiter (1996) *Clin Cancer Res* 2:245-52). The single chain antibody can be dimerized or multimerized to generate multivalent antibodies having specificities for different epitopes of the same target 26583 protein.

In a preferred embodiment the antibody has: effector function; and can fix complement. In other embodiments the antibody does not; recruit effector cells; or fix complement.

In a preferred embodiment, the antibody has reduced or no ability to bind an Fc receptor. For example., it is a isotype or subtype, fragment or other mutant, which

does not support binding to an Fc receptor, e.g., it has a mutagenized or deleted Fc receptor binding region.

The antibody can be coupled to a toxin, e.g., a polypeptide toxin, e.g., ricin or diphtheria toxin or active fragment hereof, or a radionuclide, or imaging agent, e.g. a radioactive, enzymatic, or other, e.g., imaging agent, e.g., a NMR contrast agent. Labels which produce detectable radioactive emissions or fluorescence are preferred.

An anti-26583 antibody (e.g., monoclonal antibody) can be used to isolate 26583 by standard techniques, such as affinity chromatography or immunoprecipitation. Moreover, an anti-26583 antibody can be used to detect 26583 protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the protein. Anti-26583 antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance (i.e., antibody labelling). Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase,  $\beta$ -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$  or  $^3\text{H}$ .

The invention also includes a nucleic acid that encodes an anti-26583 antibody, e.g., an anti-26583 antibody described herein. Also included are vectors which include the nucleic acid and cells transformed with the nucleic acid, particularly cells which are useful for producing an antibody, e.g., mammalian cells, e.g. CHO or lymphatic cells.

The invention also includes cell lines, e.g., hybridomas, which make an anti-26583 antibody, e.g., and antibody described herein, and method of using said cells to make a 26583 antibody.

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### Recombinant Expression Vectors, Host Cells and Genetically Engineered Cells

In another aspect, the invention includes, vectors, preferably expression vectors, containing a nucleic acid encoding a polypeptide described herein. As used  
 5 herein, the term “vector” refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked and can include a plasmid, cosmid or viral vector. The vector can be capable of autonomous replication or it can integrate into a host DNA. Viral vectors include, *e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses.

10 A vector can include a 26583 nucleic acid in a form suitable for expression of the nucleic acid in a host cell. Preferably, the recombinant expression vector includes one or more regulatory sequences operatively linked to the nucleic acid sequence to be expressed. The term “regulatory sequence” includes promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Regulatory  
 15 sequences include those that direct constitutive expression of a nucleotide sequence, as well as tissue-specific regulatory and/or inducible sequences. The design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins  
 20 or polypeptides, including fusion proteins or polypeptides, encoded by nucleic acids as described herein (*e.g.*, 26583 proteins, mutant forms of 26583 proteins, fusion proteins, and the like).

The recombinant expression vectors of the invention can be designed for expression of 26583 proteins in prokaryotic or eukaryotic cells. For example,  
 25 polypeptides of the invention can be expressed in *E. coli*, insect cells (*e.g.*, using baculovirus expression vectors), yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example  
 30 using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a

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Such fusion vectors typically serve three purposes: (1) to increase expression of recombinant protein; (2) to increase the solubility of the recombinant protein; and (3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ), which fuse glutathione S-transferase (GST), maltose E binding protein, and protein A, respectively, to the target recombinant protein.

To maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al.*, (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

A 26583 expression vector can be a yeast expression vector, a vector for expression in insect cells, *e.g.*, a baculovirus expression vector or a vector suitable for expression in mammalian cells.

When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40.

In another embodiment, the recombinant mammalian expression vector is  
 5 capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in  
 10 particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-  
 15 916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally regulated promoters are also encompassed, including for example, the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the  $\alpha$ -fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

20 The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. Regulatory sequences (*e.g.*, viral promoters and/or enhancers) operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the constitutive, tissue specific or cell type specific expression of  
 25 antisense RNA in a variety of cell types. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. *et al.*, Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

30 Another aspect the invention provides a host cell that includes a nucleic acid molecule described herein, *e.g.*, a 26583 nucleic acid molecule within a recombinant expression vector or a 26583 nucleic acid molecule containing sequences which allow it to homologously recombine into a specific site of the host cell's genome. The terms

“host cell” and “recombinant host cell” are used interchangeably herein. Such terms refer not only to the particular subject cell, but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, a 26583 protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as CHO or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into host cells via conventional transformation or transfection techniques. As used herein, the terms “transformation” and “transfection” are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation.

A host cell of the invention can be used to produce (*i.e.*, express) a 26583 protein. Accordingly, the invention further provides methods for producing a 26583 protein using the host cells of the invention. In one embodiment, the method includes culturing the host cell of the invention (into which a recombinant expression vector encoding a 26583 protein has been introduced) in a suitable medium such that a 26583 protein is produced. In another embodiment, the method further includes isolating a 26583 protein from the medium or the host cell.

In another aspect, the invention features a cell or purified preparation of cells that include a 26583 transgene, or which otherwise misexpress 26583. The cell preparation can consist of human or non-human cells, *e.g.*, rodent cells such as mouse or rat cells, rabbit cells, or pig cells. In preferred embodiments, the cell or cells include a 26583 transgene, *e.g.*, a heterologous form of a 26583 nucleic acid, *e.g.*, a gene derived from humans (in the case of a non-human cell). The 26583 transgene can be misexpressed, *e.g.*, overexpressed or underexpressed. In other preferred embodiments, the cell or cells include a gene that misexpresses an endogenous 26583 nucleic acid, *e.g.*, disruption in the expression of a gene, *e.g.*, a knockout. Such cells

can serve as a model for studying disorders that are related to mutated or mis-expressed 26583 alleles or for use in drug screening.

In another aspect, the invention features, a human cell, *e.g.*, a hematopoietic stem cell, transformed with nucleic acid that encodes a 26583 polypeptide.

5 Also provided are cells (*e.g.*, human cells, *e.g.*, a hematopoietic cell or a fibroblast cell), or a purified preparation thereof, in which an endogenous 26583 is under the control of a regulatory sequence that does not normally control the expression of the endogenous 26583 gene. The expression characteristics of an endogenous gene within a cell, *e.g.*, a cell line or microorganism, can be modified by  
10 inserting a heterologous DNA regulatory element into the genome of the cell such that the inserted regulatory element is operably linked to the endogenous 26583 gene. For example, an endogenous 26583 gene, *e.g.*, a gene that is "transcriptionally silent," *e.g.*, not normally expressed, or expressed only at very low levels, may be activated by inserting a regulatory element that is capable of promoting the expression of a  
15 normally expressed gene product in that cell. Techniques such as targeted homologous recombinations, can be used to insert the heterologous DNA as described in, *e.g.*, Chappel, US 5,272,071; WO 91/06667, published in May 16, 1991.

#### Transgenic Animals

20 The invention provides non-human transgenic animals. Such animals are useful for studying the function and/or activity of a 26583 protein and for identifying and/or evaluating modulators of 26583 activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal include a transgene.  
25 Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, and the like. A transgene is exogenous DNA or a rearrangement, *e.g.*, a deletion of endogenous chromosomal DNA, which preferably is integrated into or occurs in the genome of the cells of a transgenic animal. A transgene can direct the expression of an encoded gene product in one or more cell  
30 types or tissues of the transgenic animal, other transgenes, *e.g.*, a knockout, reduce expression. Thus, a transgenic animal can be one in which an endogenous 26583 gene has been altered by, *e.g.*, by homologous recombination between the endogenous

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gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to a transgene of the invention to direct expression of a 26583 protein to particular cells. A transgenic founder animal can be identified based upon the presence of a 26583 transgene in its genome and/or expression of 26583 mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding a 26583 protein can further be bred to other transgenic animals carrying other transgenes.

26583 proteins or polypeptides can be expressed in transgenic animals or plants, *e.g.*, a nucleic acid encoding the protein or polypeptide can be introduced into the genome of an animal. In preferred embodiments the nucleic acid is placed under the control of a tissue specific promoter, *e.g.*, a milk or egg specific promoter, and recovered from the milk or eggs produced by the animal. Suitable animals are mice, pigs, cows, goats, and sheep.

The invention also includes a population of cells from a transgenic animal, as discussed herein.

### Uses

The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: (a) screening assays; (b) predictive medicine (*e.g.*, diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenetics); and (c) methods of treatment (*e.g.*, therapeutic and prophylactic). The isolated nucleic acid molecules of the invention can be used, for example, to express a 26583 protein (*e.g.*, via a recombinant expression vector in a host cell in gene therapy applications), to detect a 26583 mRNA (*e.g.*, in a biological sample) or a genetic alteration in a 26583 gene, and to modulate 26583 activity, as described further below. The 26583 proteins can be used to treat disorders characterized by insufficient or excessive production of a 26583 substrate or production of 26583 inhibitors. In addition, the 26583 proteins can be used to screen for naturally occurring 26583 substrates, to screen for drugs or compounds that

modulate 26583 activity, as well as to treat disorders characterized by insufficient or excessive production of 26583 protein or production of 26583 protein forms which have decreased, aberrant or unwanted activity compared to 26583 wild type protein (*e.g.*, imbalance of protein serine/threonine kinase and protein serine/threonine phosphorylase activities, leading to an increase or decrease in lipid biosynthesis, such as cholesterol or cell cycle progression and neoplastic transformation). Moreover, the anti-26583 antibodies of the invention can be used to detect and isolate 26583 proteins, regulate the bioavailability of 26583 proteins, and modulate 26583 activity.

A method of evaluating a compound for the ability to interact with, *e.g.*, bind, a subject 26583 polypeptide is provided. The method includes: contacting the compound with the subject 26583 polypeptide; and evaluating ability of the compound to interact with, *e.g.*, to bind or form a complex with the subject 26583 polypeptide. This method can be performed *in vitro*, *e.g.*, in a cell free system, or *in vivo*, *e.g.*, in a two-hybrid interaction trap assay. This method can be used to identify naturally occurring molecules that interact with a subject 26583 polypeptide. It can also be used to find natural or synthetic inhibitors of a subject 26583 polypeptide. Screening methods are discussed in more detail below.

#### Screening Assays:

The invention provides methods (also referred to herein as “screening assays”) for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, proteins, peptides, peptidomimetics, peptoids, small molecules or other drugs) that bind to 26583 proteins, have a stimulatory or inhibitory effect on, for example, 26583 expression or 26583 activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of a 26583 substrate. Compounds thus identified can be used to modulate the activity of target gene products (*e.g.*, 26583 genes) in a therapeutic protocol, to elaborate the biological function of the target gene product, or to identify compounds that disrupt normal target gene interactions.

In one embodiment, the invention provides assays for screening candidate or test compounds that are substrates of a 26583 protein or polypeptide or a biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds that bind to or modulate the activity of a 26583 protein or polypeptide or a biologically active portion thereof.



The ability of the test compound to modulate 26583 binding to a compound, *e.g.*, a 26583 substrate, or to bind to 26583 can also be evaluated. This can be accomplished, for example, by coupling the compound, *e.g.*, the substrate with a radioisotope or enzymatic label such that binding of the compound, *e.g.*, the substrate, to 26583 can be determined by detecting the labeled compound, *e.g.*, substrate, in a complex. Alternatively, 26583 can be coupled with a radioisotope or enzymatic label to monitor the ability of a test compound to modulate 26583 binding to a 26583 substrate in a complex. For example, compounds (*e.g.*, 26583 substrates) can be labeled with <sup>125</sup>I, <sup>35</sup>S, <sup>14</sup>C, or <sup>3</sup>H, either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

The ability of a compound (*e.g.*, a 26583 substrate) to interact with 26583 with or without the labeling of any of the interactants can be evaluated. For example, a microphysiometer can be used to detect the interaction of a compound with 26583 without the labeling of either the compound or 26583. McConnell, H. M. *et al.* (1992) *Science* 257:1906-1912. As used herein, a "microphysiometer" (*e.g.*, Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and 26583.

In yet another embodiment, a cell-free assay is provided in which a 26583 protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the 26583 protein or biologically active portion thereof is evaluated. Preferred biologically active portions of the 26583 proteins to be used in assays of the present invention include fragments that participate in interactions with non-26583 molecules, *e.g.*, fragments with high surface probability scores.

Soluble and/or membrane-bound forms of isolated proteins (*e.g.*, 26583 proteins or biologically active portions thereof) can be used in the cell-free assays of the invention. When membrane-bound forms of the protein are used, it may be desirable to utilize a solubilizing agent. Examples of such solubilizing agents include

non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®, Isotridecypoly(ethylene glycol ether)<sub>n</sub>, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl=N,N-dimethyl-3-ammonio-1-propane sulfonate.

Cell-free assays involve preparing a reaction mixture of the target gene protein and the test compound under conditions and for a time sufficient to allow the two components to interact and bind, thus forming a complex that can be removed and/or detected.

Assay where ability of agent to block binding of serine/threonine phosphatase activity within a cell is evaluated.

The interaction between two molecules can also be detected, *e.g.*, using fluorescence energy transfer (FET) (see, for example, Lakowicz *et al.*, U.S. Patent No. 5,631,169; Stavrianopoulos, *et al.*, U.S. Patent No. 4,868,103). A fluorophore label on the first, 'donor' molecule is selected such that its emitted fluorescent energy will be absorbed by a fluorescent label on a second, 'acceptor' molecule, which in turn is able to fluoresce due to the absorbed energy. Alternately, the 'donor' protein molecule may simply utilize the natural fluorescent energy of tryptophan residues. Labels are chosen that emit different wavelengths of light, such that the 'acceptor' molecule label may be differentiated from that of the 'donor'. Since the efficiency of energy transfer between the labels is related to the distance separating the molecules, the spatial relationship between the molecules can be assessed. In a situation in which binding occurs between the molecules, the fluorescent emission of the 'acceptor' molecule label in the assay should be maximal. An FET binding event can be conveniently measured through standard fluorometric detection means well known in the art (*e.g.*, using a fluorimeter).

In another embodiment, determining the ability of the 26583 protein to bind to a target molecule can be accomplished using real-time Biomolecular Interaction Analysis (BIA) (see, *e.g.*, Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo *et al.* (1995) *Curr. Opin. Struct. Biol.* 5:699-705). "Surface plasmon resonance" or "BIA" detects biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore). Changes in the mass at the binding

surface (indicative of a binding event) result in alterations of the refractive index of light near the surface (the optical phenomenon of surface plasmon resonance (SPR)), resulting in a detectable signal that can be used as an indication of real-time reactions between biological molecules.

5 In one embodiment, the target gene product or the test substance is anchored onto a solid phase. The target gene product/test compound complexes anchored on the solid phase can be detected at the end of the reaction. Preferably, the target gene product can be anchored onto a solid surface, and the test compound (which is not anchored), can be labeled, either directly or indirectly, with detectable labels  
10 discussed herein.

It may be desirable to immobilize either 26583, an anti 26583 antibody or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a 26583 protein, or interaction of a 26583 protein with a target  
15 molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/26583 fusion  
20 proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or 26583 protein, and the mixture incubated under conditions conducive to complex formation (*e.g.*, at  
25 physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of 26583 binding or activity determined using standard  
30 techniques.

Other techniques for immobilizing either a 26583 protein or a target molecule on matrices include using conjugation of biotin and streptavidin. Biotinylated 26583 protein or target molecules can be prepared from biotin-NHS (N-hydroxy-

succinimide) using techniques known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical).

In order to conduct the assay, the non-immobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (*e.g.*, by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the previously non-immobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously non-immobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; *e.g.*, using a labeled antibody specific for the immobilized component (the antibody, in turn, can be directly labeled or indirectly labeled with, *e.g.*, a labeled anti-Ig antibody).

In one embodiment, this assay is performed utilizing antibodies reactive with 26583 protein or target molecules but which do not interfere with binding of the 26583 protein to its target molecule. Such antibodies can be derivatized to the wells of the plate, and unbound target or 26583 protein is trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the 26583 protein or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the 26583 protein or target molecule.

Alternatively, cell free assays can be conducted in a liquid phase. In such an assay, the reaction products are separated from unreacted components by any of a number of standard techniques, including but not limited to: differential centrifugation (see, for example, Rivas, G., and Minton, A.P., (1993) *Trends Biochem Sci* Aug;18(8):284-7); chromatography (gel filtration chromatography, ion-exchange chromatography); electrophoresis (see, *e.g.*, Ausubel, F. *et al.*, eds. *Current Protocols in Molecular Biology* 1999, J. Wiley: New York.); and immunoprecipitation (see, for example, Ausubel, F. *et al.*, eds. *Current Protocols in Molecular Biology* 1999, J. Wiley: New York). Such resins and chromatographic techniques are known to one skilled in the art (see, *e.g.*, Heegaard, N.H., (1998) *J Mol Recognit* Winter;11(1-

6):141-8; Hage, D.S., and Tweed, S.A. (1997) *J. Chromatogr B. Biomed Sci Appl* Oct 10;699(1-2):499-525). Further, fluorescence energy transfer may also be conveniently utilized, as described herein, to detect binding without further purification of the complex from solution.

5 In a preferred embodiment, the assay includes contacting the 26583 protein or biologically active portion thereof with a known compound which binds 26583 to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a 26583 protein, wherein determining the ability of the test compound to interact with a 26583 protein includes  
10 determining the ability of the test compound to preferentially bind to 26583 or biologically active portion thereof, or to modulate the activity of a target molecule, as compared to the known compound.

The target gene products of the invention can, *in vivo*, interact with one or more cellular or extracellular macromolecules, such as proteins. For the purposes of  
15 this discussion, such cellular and extracellular macromolecules are referred to herein as "binding partners." Compounds that disrupt such interactions can be useful in regulating the activity of the target gene product. Such compounds can include, but are not limited to molecules such as antibodies, peptides, and small molecules. The preferred target genes/products for use in this embodiment are the 26583 genes herein  
20 identified. In an alternative embodiment, the invention provides methods for determining the ability of the test compound to modulate the activity of a 26583 protein through modulation of the activity of a downstream effector of a 26583 target molecule. For example, the activity of the effector molecule on an appropriate target can be determined, or the binding of the effector to an appropriate target can be  
25 determined, as previously described.

To identify compounds that interfere with the interaction between the target gene product and its cellular or extracellular binding partner(s), *e.g.*, a substrate, a reaction mixture containing the target gene product and the binding partner is prepared, under conditions and for a time sufficient, to allow the two products to form  
30 complex. In order to test an inhibitory agent, the reaction mixture is provided in the presence and absence of the test compound. The test compound can be initially included in the reaction mixture, or can be added at a time subsequent to the addition of the target gene and its cellular or extracellular binding partner. Control reaction



mixtures are incubated without the test compound or with a placebo. The formation of any complexes between the target gene product and the cellular or extracellular binding partner is then detected. The formation of a complex in the control reaction, but not in the reaction mixture containing the test compound, indicates that the compound interferes with the interaction of the target gene product and the interactive binding partner. Additionally, complex formation within reaction mixtures containing the test compound and normal target gene product can also be compared to complex formation within reaction mixtures containing the test compound and mutant target gene product. This comparison can be important in those cases wherein it is desirable to identify compounds that disrupt interactions of mutant but not normal target gene products.

These assays can be conducted in a heterogeneous or homogeneous format. Heterogeneous assays involve anchoring either the target gene product or the binding partner onto a solid phase, and detecting complexes anchored on the solid phase at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. In either approach, the order of addition of reactants can be varied to obtain different information about the compounds being tested. For example, test compounds that interfere with the interaction between the target gene products and the binding partners, *e.g.*, by competition, can be identified by conducting the reaction in the presence of the test substance. Alternatively, test compounds that disrupt preformed complexes, *e.g.*, compounds with higher binding constants that displace one of the components from the complex, can be tested by adding the test compound to the reaction mixture after complexes have been formed. The various formats are briefly described below.

In a heterogeneous assay system, either the target gene product or the interactive cellular or extracellular binding partners, is anchored onto a solid surface (*e.g.*, a microtiter plate), while the non-anchored species is labeled either directly or indirectly. The anchored species can be immobilized by non-covalent or covalent attachments. Alternatively, an immobilized antibody specific for the species to be anchored can be used to anchor the species to the solid surface.

In order to conduct the assay, the partner of the immobilized species is exposed to the coated surface with or without the test compound. After the reaction is complete, unreacted components are removed (*e.g.*, by washing) and any complexes

that have formed remain immobilized on the solid surface. In assays where the non-immobilized species is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. In assays where the non-immobilized species is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; *e.g.*, using a labeled antibody specific for the initially non-immobilized species (the antibody, in turn, can be directly labeled or indirectly labeled with, *e.g.*, a labeled anti-Ig antibody). Depending upon the order of addition of reaction components, test compounds that inhibit complex formation or that disrupt preformed complexes can be detected.

Alternatively, the reaction can be conducted in a liquid phase in the presence or absence of the test compound. Reaction products are separated from unreacted components and complexes detected using, for example, an immobilized antibody specific for one of the binding components to anchor any complexes formed in solution and a labeled antibody specific for the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the liquid phase, test compounds that inhibit complex formation or that disrupt preformed complexes can be identified.

In an alternate embodiment of the invention, a homogeneous assay can be used. For example, a preformed complex of the target gene product and the interactive cellular or extracellular binding partner product is prepared in which either the target gene products or their binding partners are labeled, but the signal generated by the label is quenched due to complex formation (see, *e.g.*, U.S. Patent No. 4,109,496 that utilizes this approach for immunoassays). The addition of a test substance that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above background. In this way, test substances that disrupt target gene product-binding partner interaction can be identified.

In yet another aspect, the 26583 proteins can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J. Biol. Chem.* 268:12046-12054; Bartel *et al.* (1993) *Biotechniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact with 26583 ("26583-binding proteins" or "26583-bp") and are involved in

26583 activity. Such 26583-bps can be activators or inhibitors of signals by the 26583 proteins or 26583 targets as, for example, downstream elements of a 26583-mediated signaling pathway.

The two-hybrid system is based on the modular nature of most transcription  
5 factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a 26583 protein is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence from a library of DNA sequences that encodes an unidentified protein (“prey” or “sample”) is  
10 fused to a gene that codes for the activation domain of the known transcription factor. (Alternatively the 26583 protein can be fused to the activator domain.) If the “bait” and the “prey” proteins are able to interact *in vivo* and form a 26583-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene  
15 (*e.g.*, LacZ) that is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene that encodes the protein that interacts with the 26583 protein.

In another embodiment, modulators of 26583 expression are identified. For  
20 example, a cell or cell free mixture is contacted with a candidate compound and the expression of 26583 mRNA or protein evaluated relative to the level of expression of 26583 mRNA or protein in the absence of the candidate compound. When expression of 26583 mRNA or protein is greater in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of 26583 mRNA  
25 or protein expression. Alternatively, when expression of 26583 mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of 26583 mRNA or protein expression. The level of 26583 mRNA or protein expression can be determined by methods described herein for detecting 26583 mRNA or protein.

30 In another aspect, the invention pertains to a combination of two or more of the assays described herein. For example, a modulating agent can be identified using a cell-based or a cell free assay, and the ability of the agent to modulate the activity of

a 26583 protein can be confirmed *in vivo*, *e.g.*, in an animal such as an animal model overexpressing a gene encoding a protein serine/threonine kinase.

This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein (*e.g.*, a 26583 modulating agent, an antisense 26583 nucleic acid molecule, a 26583-specific antibody, or a 26583-binding partner) in an appropriate animal model to determine the efficacy, toxicity, side effects, or mechanism of action, of treatment with such an agent. Furthermore, novel agents identified by the above-described screening assays can be used for treatments as described herein.

#### Detection Assays

Portions or fragments of the nucleic acid sequences identified herein can be used as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome *e.g.*, to locate gene regions associated with genetic disease or to associate 26583 with a disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

#### Chromosome Mapping

The 26583 nucleotide sequences or portions thereof can be used to map the location of the 26583 genes on a chromosome. This process is called chromosome mapping. Chromosome mapping is useful in correlating the 26583 sequences with genes associated with disease.

Briefly, 26583 genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the 26583 nucleotide sequences. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the 26583 sequences will yield an amplified fragment.

A panel of somatic cell hybrids in which each cell line contains either a single human chromosome or a small number of human chromosomes and a full set of mouse chromosomes, allows easy mapping of individual genes to specific human chromosomes. (D'Eustachio P. *et al.* (1983) *Science* 220:919-924).

Other mapping strategies *e.g.*, *in situ* hybridization (described in Fan, Y. *et al.* (1990) *Proc. Natl. Acad. Sci. USA*, 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries can be used to map 26583 to a chromosomal location.

5        Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple  
10       detection. Preferably 1,000 bases, and more preferably 2,000 bases will suffice to get good results at a reasonable amount of time. For a review of this technique, see Verma *et al.*, Human Chromosomes: A Manual of Basic Techniques (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single  
15       chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

20       Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man, available on-line through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal  
25       region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland, J. *et al.* (1987) *Nature*, 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the 26583 gene, can be determined. If a  
30       mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions

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or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

5

#### Tissue Typing

26583 sequences can be used to identify individuals from biological samples using, *e.g.*, restriction fragment length polymorphism (RFLP). In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, the  
10 fragments separated, *e.g.*, by electrophoresis and Southern blotted, and probed to yield bands for identification. The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

Furthermore, the sequences of the present invention can also be used to determine the actual base-by-base DNA sequence of selected portions of an  
15 individual's genome. Thus, the 26583 nucleotide sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it. Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a  
20 unique set of such DNA sequences due to allelic differences.

Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers  
25 of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NO:1 can provide positive individual identification with a panel of perhaps 10 to 1,000 primers, which each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO:3 are used, a more appropriate number of  
30 primers for positive individual identification would be 500-2,000.

If a panel of reagents from 26583 nucleotide sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique

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identification database, positive identification of the individual, living or dead, can be made from extremely small tissue samples.

#### Use of Partial 26583 Sequences in Forensic Biology

5 DNA-based identification techniques can also be used in forensic biology. To make such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, *e.g.*, hair or skin, or body fluids, *e.g.*, blood, saliva, or semen, found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of  
10 the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, *e.g.*, PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (*i.e.*, another DNA sequence that is unique  
15 to a particular individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to noncoding regions of SEQ ID NO:1 (*e.g.*, fragments derived from the noncoding regions of SEQ ID NO:1 and having a length of at least 20 bases, preferably at least 30 bases) are particularly  
20 appropriate for this use.

The 26583 nucleotide sequences described herein can further be used to provide polynucleotide reagents, *e.g.*, labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue, *e.g.*, a tissue containing 26583 serine/threonine phosphatase activity. This can be very  
25 useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such 26583 probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, *e.g.*, 26583 primers or probes can be used to screen tissue culture for contamination (*i.e.*, screen for the presence of a mixture of  
30 different types of cells in a culture).

#### Predictive Medicine

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual.

Generally, the invention provides, a method of determining if a subject is at  
 5 risk for a disorder related to a lesion in or the misexpression of a gene that encodes  
 26583. Such disorders include, *e.g.*, a disorder associated with the misexpression of  
 26583.

The method includes one or more of the following:

detecting, in a tissue of the subject, the presence or absence of a  
 10 mutation which affects the expression of the 26583 gene, or detecting the  
 presence or absence of a mutation in a region which controls the expression of  
 the gene, *e.g.*, a mutation in the 5' control region;

detecting, in a tissue of the subject, the presence or absence of a  
 mutation which alters the structure of the 26583 gene;

15 detecting, in a tissue of the subject, the misexpression of the 26583  
 gene at the mRNA level, *e.g.*, detecting a non-wild type level of a mRNA ;

detecting, in a tissue of the subject, the misexpression of the gene at the  
 protein level, *e.g.*, detecting a non-wild type level of a 26583 polypeptide.

In preferred embodiments the method includes: ascertaining the existence of at  
 20 least one of: a deletion of one or more nucleotides from the 26583 gene; an insertion  
 of one or more nucleotides into the gene, a point mutation, *e.g.*, a substitution of one  
 or more nucleotides of the gene, or a gross chromosomal rearrangement of the gene,  
*e.g.*, a translocation, inversion, or deletion.

For example, detecting the genetic lesion can include: (i) providing a  
 25 probe/primer including an oligonucleotide containing a region of nucleotide sequence  
 that hybridizes to a sense or antisense sequence from SEQ ID NO:1 or 3, or naturally  
 occurring mutants thereof or 5' or 3' flanking sequences naturally associated with the  
 26583 gene; (ii) exposing the probe/primer to nucleic acid of the tissue; and (iii)  
 detecting, by hybridization, *e.g.*, *in situ* hybridization, of the probe/primer to the  
 30 nucleic acid, the presence or absence of the genetic lesion.

In preferred embodiments detecting the misexpression includes ascertaining  
 the existence of at least one of: an alteration in the level of a messenger RNA

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transcript of the 26583 gene; the presence of a non-wild type splicing pattern of a messenger RNA transcript of the gene; or a non-wild type level of 26583.

Methods of the invention can be used prenatally or to determine if a subject's offspring will be at risk for a disorder.

5 In preferred embodiments the method includes determining the structure of a 26583 gene, an abnormal structure being indicative of risk for the disorder.

In preferred embodiments the method includes contacting a sample from the subject with an antibody to the 26583 protein or a nucleic acid, which hybridizes specifically with the gene. This and other embodiments are discussed below.

10

#### Diagnostic and Prognostic Assays

Diagnostic and prognostic assays of the invention include method for assessing the expression level of 26583 molecules and for identifying variations and mutations in the sequence of 26583 molecules.

15

#### *Expression Monitoring and Profiling.*

The presence, level, or absence of 26583 protein or nucleic acid in a biological sample can be evaluated by obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting 20 26583 protein or nucleic acid (e.g., mRNA, genomic DNA) that encodes 26583 protein such that the presence of 26583 protein or nucleic acid is detected in the biological sample. The term "biological sample" includes tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. A preferred biological sample is serum. The level of expression of the 26583 gene can be measured in a number of ways, including, but not limited to: measuring 25 the mRNA encoded by the 26583 genes; measuring the amount of protein encoded by the 26583 genes; or measuring the activity of the protein encoded by the 26583 genes.

The level of mRNA corresponding to the 26583 gene in a cell can be determined both by *in situ* and by *in vitro* formats.

30

The isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or Northern analyses, polymerase chain reaction analyses and probe arrays. One preferred diagnostic method for the detection of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule

(probe) that can hybridize to the mRNA encoded by the gene being detected. The nucleic acid probe can be, for example, a full-length 26583 nucleic acid, such as the nucleic acid of SEQ ID NO:1, or a portion thereof, such as an oligonucleotide of at least 7, 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to 26583 mRNA or genomic DNA. The probe can be disposed on an address of an array, e.g., an array described below. Other suitable probes for use in the diagnostic assays are described herein.

In one format, mRNA (or cDNA) is immobilized on a surface and contacted with the probes, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative format, the probes are immobilized on a surface and the mRNA (or cDNA) is contacted with the probes, for example, in a two-dimensional gene chip array described below. A skilled artisan can adapt known mRNA detection methods for use in detecting the level of mRNA encoded by the 26583 genes.

The level of mRNA in a sample that is encoded by one of 26583 can be evaluated with nucleic acid amplification, e.g., by rtPCR (Mullis (1987) U.S. Patent No. 4,683,202), ligase chain reaction (Barany (1991) *Proc. Natl. Acad. Sci. USA* 88:189-193), self sustained sequence replication (Guatelli *et al.*, (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh *et al.*, (1989), *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi *et al.*, (1988) *Bio/Technology* 6:1197), rolling circle replication (Lizardi *et al.*, U.S. Patent No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques known in the art. As used herein, amplification primers are defined as being a pair of nucleic acid molecules that can anneal to 5' or 3' regions of a gene (plus and minus strands, respectively, or vice-versa) and contain a short region in between. In general, amplification primers are from about 10 to 30 nucleotides in length and flank a region from about 50 to 200 nucleotides in length. Under appropriate conditions and with appropriate reagents, such primers permit the amplification of a nucleic acid molecule comprising the nucleotide sequence flanked by the primers.

For *in situ* methods, a cell or tissue sample can be prepared/processed and immobilized on a support, typically a glass slide, and then contacted with a probe that can hybridize to mRNA that encodes the 26583 gene being analyzed.

In another embodiment, the methods further contacting a control sample with a compound or agent capable of detecting 26583 mRNA, or genomic DNA, and comparing the presence of 26583 mRNA or genomic DNA in the control sample with the presence of 26583 mRNA or genomic DNA in the test sample. In still another  
5 embodiment, serial analysis of gene expression, as described in U.S. Patent No. 5,695,937, is used to detect 26583 transcript levels.

A variety of methods can be used to determine the level of protein encoded by 26583. In general, these methods include contacting an agent that selectively binds to the protein, such as an antibody with a sample, to evaluate the level of protein in the  
10 sample. In a preferred embodiment, the antibody bears a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')<sub>2</sub>) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody,  
15 as well as indirect labeling of the probe or antibody by reactivity with a detectable substance. Examples of detectable substances are provided herein.

The detection methods can be used to detect 26583 protein in a biological sample *in vitro* as well as *in vivo*. *In vitro* techniques for detection of 26583 protein include enzyme linked immunosorbent assays (ELISAs), immunoprecipitations,  
20 immunofluorescence, enzyme immunoassay (EIA), radioimmunoassay (RIA), and Western blot analysis. *In vivo* techniques for detection of 26583 protein include introducing into a subject a labeled anti-26583 antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques. In another embodiment, the sample is  
25 labeled, e.g., biotinylated and then contacted to the antibody, e.g., an anti-26583 antibody positioned on an antibody array (as described below). The sample can be detected, e.g., with avidin coupled to a fluorescent label.

In another embodiment, the methods further include contacting the control sample with a compound or agent capable of detecting 26583 protein, and comparing  
30 the presence of 26583 protein in the control sample with the presence of 26583 protein in the test sample.

The invention also includes kits for detecting the presence of 26583 in a biological sample. For example, the kit can include a compound or agent capable of

detecting 26583 protein or mRNA in a biological sample; and a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect 26583 protein or nucleic acid.

For antibody-based kits, the kit can include: (1) a first antibody (e.g., attached to a solid support) which binds to a polypeptide corresponding to a marker of the invention; and, optionally, (2) a second, different antibody which binds to either the polypeptide or the first antibody and is conjugated to a detectable agent.

For oligonucleotide-based kits, the kit can include: (1) an oligonucleotide, e.g., a detectably labeled oligonucleotide, which hybridizes to a nucleic acid sequence encoding a polypeptide corresponding to a marker of the invention or (2) a pair of primers useful for amplifying a nucleic acid molecule corresponding to a marker of the invention. The kit can also include a buffering agent, a preservative, or a protein stabilizing agent. The kit can also include components necessary for detecting the detectable agent (e.g., an enzyme or a substrate). The kit can also contain a control sample or a series of control samples which can be assayed and compared to the test sample contained. Each component of the kit can be enclosed within an individual container and all of the various containers can be within a single package, along with instructions for interpreting the results of the assays performed using the kit.

The diagnostic methods described herein can identify subjects having, or at risk of developing, a disease or disorder associated with misexpressed or aberrant or unwanted 26583 expression or activity. As used herein, the term "unwanted" includes an unwanted phenomenon involved in a biological response such as pain or deregulated cell proliferation.

In one embodiment, a disease or disorder associated with aberrant or unwanted 26583 expression or activity is identified. A test sample is obtained from a subject and 26583 protein or nucleic acid (e.g., mRNA or genomic DNA) is evaluated, wherein the level, e.g., the presence or absence, of 26583 protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant or unwanted 26583 expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest, including a biological fluid (e.g., serum), cell sample, or tissue.

The prognostic assays described herein can be used to determine whether a subject can be administered an agent (e.g., an agonist, antagonist, peptidomimetic,

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protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant or unwanted 26583 expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a metabolic disorder, e.g., a mitochondrial related disorder or a cholesterol biosynthesis related disorder, or a cell proliferation or differentiation disorder, e.g., a tumor.

In another aspect, the invention features a computer medium having a plurality of digitally encoded data records. Each data record includes a value representing the level of expression of 26583 in a sample, and a descriptor of the sample. The descriptor of the sample can be an identifier of the sample, a subject from which the sample was derived (e.g., a patient), a diagnosis, or a treatment (e.g., a preferred treatment). In a preferred embodiment, the data record further includes values representing the level of expression of genes other than 26583 (e.g., other genes associated with a 26583-disorder, or other genes on an array). The data record can be structured as a table, e.g., a table that is part of a database such as a relational database (e.g., a SQL database of the Oracle or Sybase database environments).

Also featured is a method of evaluating a sample. The method includes providing a sample, e.g., from the subject, and determining a gene expression profile of the sample, wherein the profile includes a value representing the level of 26583 expression. The method can further include comparing the value or the profile (i.e., multiple values) to a reference value or reference profile. The gene expression profile of the sample can be obtained by any of the methods described herein (e.g., by providing a nucleic acid from the sample and contacting the nucleic acid to an array). The method can be used to diagnose a metabolic disorder, e.g., a mitochondrial related disorder or a cholesterol biosynthesis related disorder, or a cell proliferation or differentiation disorder, e.g., a tumor, in a subject wherein altered 26583 expression is an indication that the subject has or is disposed to having a metabolic disorder, e.g., a mitochondrial related disorder or a cholesterol biosynthesis related disorder, or a cell proliferation or differentiation disorder, e.g., a tumor. The method can be used to monitor a treatment for a metabolic disorder, e.g., a mitochondrial related disorder or a cholesterol biosynthesis related disorder, or a cell proliferation or differentiation disorder, e.g., a tumor. For example, the gene expression profile can be determined for a sample from a subject undergoing treatment. The profile can be compared to a

reference profile or to a profile obtained from the subject prior to treatment or prior to onset of the disorder (see, e.g., Golub *et al.* (1999) *Science* 286:531).

In yet another aspect, the invention features a method of evaluating a test compound (see also, "Screening Assays", above). The method includes providing a  
5 cell and a test compound; contacting the test compound to the cell; obtaining a subject expression profile for the contacted cell; and comparing the subject expression profile to one or more reference profiles. The profiles include a value representing the level of 26583 expression. In a preferred embodiment, the subject expression profile is compared to a target profile, e.g., a profile for a normal cell or for desired condition of  
10 a cell. The test compound is evaluated favorably if the subject expression profile is more similar to the target profile than an expression profile obtained from an uncontacted cell.

In another aspect, the invention features, a method of evaluating a subject. The method includes: a) obtaining a sample from a subject, e.g., from a caregiver,  
15 e.g., a caregiver who obtains the sample from the subject; b) determining a subject expression profile for the sample. Optionally, the method further includes either or both of steps: c) comparing the subject expression profile to one or more reference expression profiles; and d) selecting the reference profile most similar to the subject reference profile. The subject expression profile and the reference profiles include a  
20 value representing the level of 26583 expression. A variety of routine statistical measures can be used to compare two reference profiles. One possible metric is the length of the distance vector that is the difference between the two profiles. Each of the subject and reference profile is represented as a multi-dimensional vector, wherein each dimension is a value in the profile.

25 The method can further include transmitting a result to a caregiver. The result can be the subject expression profile, a result of a comparison of the subject expression profile with another profile, a most similar reference profile, or a descriptor of any of the aforementioned. The result can be transmitted across a computer network, e.g., the result can be in the form of a computer transmission, e.g.,  
30 a computer data signal embedded in a carrier wave.

Also featured is a computer medium having executable code for effecting the following steps: receive a subject expression profile; access a database of reference expression profiles; and either i) select a matching reference profile most similar to

the subject expression profile or ii) determine at least one comparison score for the similarity of the subject expression profile to at least one reference profile. The subject expression profile, and the reference expression profiles each include a value representing the level of 26583 expression.

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#### Arrays and Uses Thereof

In another aspect, the invention features an array that includes a substrate having a plurality of addresses. At least one address of the plurality includes a capture probe that binds specifically to a 26583 molecule (e.g., a 26583 nucleic acid  
10 or a 26583 polypeptide). The array can have a density of at least 10, 50, 100, 200, 500, 1,000, 2,000, or 10,000 or more addresses/cm<sup>2</sup>, and ranges between. In a preferred embodiment, the plurality of addresses includes at least 10, 100, 500, 1,000, 5,000, 10,000, 50,000 addresses. In a preferred embodiment, the plurality of addresses includes equal to or less than 10, 100, 500, 1,000, 5,000, 10,000, or 50,000  
15 addresses. The substrate can be a two-dimensional substrate such as a glass slide, a wafer (e.g., silica or plastic), a mass spectroscopy plate, or a three-dimensional substrate such as a gel pad. Addresses in addition to address of the plurality can be disposed on the array.

In a preferred embodiment, at least one address of the plurality includes a  
20 nucleic acid capture probe that hybridizes specifically to a 26583 nucleic acid, e.g., the sense or anti-sense strand. In one preferred embodiment, a subset of addresses of the plurality of addresses has a nucleic acid capture probe for 26583. Each address of the subset can include a capture probe that hybridizes to a different region of a 26583 nucleic acid. In another preferred embodiment, addresses of the subset include a  
25 capture probe for a 26583 nucleic acid. Each address of the subset is unique, overlapping, and complementary to a different variant of 26583 (e.g., an allelic variant, or all possible hypothetical variants). The array can be used to sequence 26583 by hybridization (see, e.g., U.S. Patent No. 5,695,940).

An array can be generated by various methods, e.g., by photolithographic  
30 methods (see, e.g., U.S. Patent Nos. 5,143,854; 5,510,270; and 5,527,681), mechanical methods (e.g., directed-flow methods as described in U.S. Patent No.

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5,384,261), pin-based methods (e.g., as described in U.S. Pat. No. 5,288,514), and bead-based techniques (e.g., as described in PCT US/93/04145).

In another preferred embodiment, at least one address of the plurality includes a polypeptide capture probe that binds specifically to a 26583 polypeptide or fragment thereof. The polypeptide can be a naturally-occurring interaction partner of 26583 polypeptide. Preferably, the polypeptide is an antibody, e.g., an antibody described herein (see "Anti-26583 Antibodies," above), such as a monoclonal antibody or a single-chain antibody.

In another aspect, the invention features a method of analyzing the expression of 26583. The method includes providing an array as described above; contacting the array with a sample and detecting binding of a 26583-molecule (e.g., nucleic acid or polypeptide) to the array. In a preferred embodiment, the array is a nucleic acid array. Optionally the method further includes amplifying nucleic acid from the sample prior or during contact with the array.

In another embodiment, the array can be used to assay gene expression in a tissue to ascertain tissue specificity of genes in the array, particularly the expression of 26583. If a sufficient number of diverse samples is analyzed, clustering (e.g., hierarchical clustering, k-means clustering, Bayesian clustering and the like) can be used to identify other genes which are co-regulated with 26583. For example, the array can be used for the quantitation of the expression of multiple genes. Thus, not only tissue specificity, but also the level of expression of a battery of genes in the tissue is ascertained. Quantitative data can be used to group (e.g., cluster) genes on the basis of their tissue expression *per se* and level of expression in that tissue.

For example, array analysis of gene expression can be used to assess the effect of cell-cell interactions on 26583 expression. A first tissue can be perturbed and nucleic acid from a second tissue that interacts with the first tissue can be analyzed. In this context, the effect of one cell type on another cell type in response to a biological stimulus can be determined, e.g., to monitor the effect of cell-cell interaction at the level of gene expression.

In another embodiment, cells are contacted with a therapeutic agent. The expression profile of the cells is determined using the array, and the expression profile is compared to the profile of like cells not contacted with the agent. For example, the assay can be used to determine or analyze the molecular basis of an undesirable effect



of the therapeutic agent. If an agent is administered therapeutically to treat one cell type but has an undesirable effect on another cell type, the invention provides an assay to determine the molecular basis of the undesirable effect and thus provides the opportunity to co-administer a counteracting agent or otherwise treat the undesired effect. Similarly, even within a single cell type, undesirable biological effects can be determined at the molecular level. Thus, the effects of an agent on expression of other than the target gene can be ascertained and counteracted.

In another embodiment, the array can be used to monitor expression of one or more genes in the array with respect to time. For example, samples obtained from different time points can be probed with the array. Such analysis can identify and/or characterize the development of a 26583-associated disease or disorder; and processes, such as a cellular transformation associated with a 26583-associated disease or disorder. The method can also evaluate the treatment and/or progression of a 26583-associated disease or disorder

The array is also useful for ascertaining differential expression patterns of one or more genes in normal and abnormal cells. This provides a battery of genes (e.g., including 26583) that could serve as a molecular target for diagnosis or therapeutic intervention.

In another aspect, the invention features an array having a plurality of addresses. Each address of the plurality includes a unique polypeptide. At least one address of the plurality has disposed thereon a 26583 polypeptide or fragment thereof. Methods of producing polypeptide arrays are described in the art, e.g., in De Wildt *et al.* (2000). *Nature Biotech.* 18, 989-994; Lueking *et al.* (1999). *Anal. Biochem.* 270, 103-111; Ge, H. (2000). *Nucleic Acids Res.* 28, e3, I-VII; MacBeath, G., and Schreiber, S.L. (2000). *Science* 289, 1760-1763; and WO 99/51773A1. In a preferred embodiment, each addresses of the plurality has disposed thereon a polypeptide at least 60, 70, 80, 85, 90, 95 or 99 % identical to a 26583 polypeptide or fragment thereof. For example, multiple variants of a 26583 polypeptide (e.g., encoded by allelic variants, site-directed mutants, random mutants, or combinatorial mutants) can be disposed at individual addresses of the plurality. Addresses in addition to the address of the plurality can be disposed on the array.

The array is also useful for ascertaining the effect of the expression of a gene on the expression of other genes in the same cell or in different cells (*e.g.*, ascertaining the effect of 26583 expression on the expression of other genes). This provides, for example, for a selection of alternate molecular targets for therapeutic intervention if the ultimate or downstream target cannot be regulated.

In another aspect, the invention features a method of analyzing a plurality of probes or a sample. The method is useful, e.g., for analyzing gene expression. The method includes: providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality having a unique capture probe, contacting the array with a first

25                    Detection of Variations or Mutations

The methods of the invention can also be used to detect genetic alterations in a 26583 gene, thereby determining if a subject with the altered gene is at risk for a disorder characterized by misregulation in 26583 protein activity or nucleic acid expression, such as a metabolic disorder, e.g., a mitochondrial related disorder or a 30 cholesterol biosynthesis related disorder, or a cell proliferation or differentiation disorder, e.g., a tumor. In preferred embodiments, the methods include detecting, in a sample from the subject, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding a 26583-

protein, or the mis-expression of the 26583 gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from a 26583 gene; 2) an addition of one or more nucleotides to a 26583 gene; 3) a substitution of one or more nucleotides of a 26583 gene, 4) a chromosomal rearrangement of a 26583 gene; 5) an alteration in the level of a messenger RNA transcript of a 26583 gene, 6) aberrant modification of a 26583 gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a 26583 gene, 8) a non-wild type level of a 26583-protein, 9) allelic loss of a 26583 gene, and 10) inappropriate post-translational modification of a 26583-protein.

An alteration can be detected without a probe/primer in a polymerase chain reaction, such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR), the latter of which can be particularly useful for detecting point mutations in the 26583-gene. This method can include the steps of collecting a sample of cells from a subject, isolating nucleic acid (e.g., genomic, mRNA or both) from the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a 26583 gene under conditions such that hybridization and amplification of the 26583-gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein. Alternatively, other amplification methods described herein or known in the art can be used.

In another embodiment, mutations in a 26583 gene from a sample cell can be identified by detecting alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined, e.g., by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the 26583 gene and detect mutations by comparing the sequence of the sample 26583 with the corresponding wild-type (control) sequence. Automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Biotechniques* 19:448), including sequencing by mass spectrometry.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so

called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in 26583 cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662; U.S. Patent No. 5,459,039).

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in 26583 genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci USA*: 86:2766, see also Cotton (1993) *Mutat. Res.* 285:125-144; and Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control 26583 nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet* 7:5).

In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl Acad. Sci USA* 86:6230). A further method of detecting point mutations is the

chemical ligation of oligonucleotides as described in Xu *et al.* ((2001) *Nature Biotechnol.* 19:148). Adjacent oligonucleotides, one of which selectively anneals to the query site, are ligated together if the nucleotide at the query site of the sample nucleic acid is complementary to the query oligonucleotide; ligation can be  
 5 monitored, e.g., by fluorescent dyes coupled to the oligonucleotides.

Alternatively, allele specific amplification technology that depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential  
 10 hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes*  
 15 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

20 In another aspect, the invention features a set of oligonucleotides. The set includes a plurality of oligonucleotides, each of which is at least partially complementary (e.g., at least 50%, 60%, 70%, 80%, 90%, 92%, 95%, 97%, 98%, or 99% complementary) to a 26583 nucleic acid.

In a preferred embodiment the set includes a first and a second  
 25 oligonucleotide. The first and second oligonucleotide can hybridize to the same or to different locations of SEQ ID NO: 1 or 3, or the complement of SEQ ID NO:1 or 3. Different locations can be different but overlapping or nonoverlapping on the same strand. The first and second oligonucleotide can hybridize to sites on the same or on different strands.

30 The set can be useful, e.g., for identifying SNP's, or identifying specific alleles of 26583. In a preferred embodiment, each oligonucleotide of the set has a different nucleotide at an interrogation position. In one embodiment, the set includes two

oligonucleotides, each complementary to a different allele at a locus, e.g., a biallelic or polymorphic locus.

In another embodiment, the set includes four oligonucleotides, each having a different nucleotide (e.g., adenine, guanine, cytosine, or thymidine) at the  
5 interrogation position. The interrogation position can be a SNP or the site of a mutation. In another preferred embodiment, the oligonucleotides of the plurality are identical in sequence to one another (except for differences in length). The oligonucleotides can be provided with differential labels, such that an oligonucleotide that hybridizes to one allele provides a signal that is distinguishable from an  
10 oligonucleotide that hybridizes to a second allele. In still another embodiment, at least one of the oligonucleotides of the set has a nucleotide change at a position in addition to a query position, e.g., a destabilizing mutation to decrease the  $T_m$  of the oligonucleotide. In another embodiment, at least one oligonucleotide of the set has a non-natural nucleotide, e.g., inosine. In a preferred embodiment, the oligonucleotides  
15 are attached to a solid support, e.g., to different addresses of an array or to different beads or nanoparticles.

In a preferred embodiment the set of oligo nucleotides can be used to specifically amplify, e.g., by PCR, or detect, a 26583 nucleic acid.

The methods described herein may be performed, for example, by utilizing  
20 pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a 26583 gene.

#### Use of 26583 Molecules as Surrogate Markers

25 The 26583 molecules of the invention are also useful as markers of disorders or disease states, as markers for precursors of disease states, as markers for predisposition of disease states, as markers of drug activity, or as markers of the pharmacogenomic profile of a subject. Using the methods described herein, the presence, absence and/or quantity of the 26583 molecules of the invention may be  
30 detected, and may be correlated with one or more biological states in vivo. For example, the 26583 molecules of the invention may serve as surrogate markers for one or more disorders or disease states or for conditions leading up to disease states.



As used herein, a “surrogate marker” is an objective biochemical marker which correlates with the absence or presence of a disease or disorder, or with the progression of a disease or disorder (e.g., with the presence or absence of a tumor). The presence or quantity of such markers is independent of the disease. Therefore, these markers may serve to indicate whether a particular course of treatment is effective in lessening a disease state or disorder. Surrogate markers are of particular use when the presence or extent of a disease state or disorder is difficult to assess through standard methodologies (e.g., early stage tumors), or when an assessment of disease progression is desired before a potentially dangerous clinical endpoint is reached (e.g., an assessment of cardiovascular disease may be made using cholesterol levels as a surrogate marker, and an analysis of HIV infection may be made using HIV RNA levels as a surrogate marker, well in advance of the undesirable clinical outcomes of myocardial infarction or fully-developed AIDS). Examples of the use of surrogate markers in the art include: Koomen *et al.* (2000) *J. Mass. Spectrom.* 35: 258-264; and James (1994) *AIDS Treatment News Archive* 209.

The 26583 molecules of the invention are also useful as pharmacodynamic markers. As used herein, a “pharmacodynamic marker” is an objective biochemical marker which correlates specifically with drug effects. The presence or quantity of a pharmacodynamic marker is not related to the disease state or disorder for which the drug is being administered; therefore, the presence or quantity of the marker is indicative of the presence or activity of the drug in a subject. For example, a pharmacodynamic marker may be indicative of the concentration of the drug in a biological tissue, in that the marker is either expressed or transcribed or not expressed or transcribed in that tissue in relationship to the level of the drug. In this fashion, the distribution or uptake of the drug may be monitored by the pharmacodynamic marker. Similarly, the presence or quantity of the pharmacodynamic marker may be related to the presence or quantity of the metabolic product of a drug, such that the presence or quantity of the marker is indicative of the relative breakdown rate of the drug in vivo. Pharmacodynamic markers are of particular use in increasing the sensitivity of detection of drug effects, particularly when the drug is administered in low doses. Since even a small amount of a drug may be sufficient to activate multiple rounds of marker (e.g., a 26583 marker) transcription or expression, the amplified marker may be in a quantity which is more readily detectable than the drug itself. Also, the

marker may be more easily detected due to the nature of the marker itself; for example, using the methods described herein, anti-26583 antibodies may be employed in an immune-based detection system for a 26583 protein marker, or 26583-specific radiolabeled probes may be used to detect a 26583 mRNA marker. Furthermore, the use of a pharmacodynamic marker may offer mechanism-based prediction of risk due to drug treatment beyond the range of possible direct observations. Examples of the use of pharmacodynamic markers in the art include: Matsuda *et al.* US 6,033,862; Hattis *et al.* (1991) *Env. Health Perspect.* 90: 229-238; Schentag (1999) *Am. J. Health-Syst. Pharm.* 56 Suppl. 3: S21-S24; and Nicolau (1999) *Am. J. Health-Syst. Pharm.* 56 Suppl. 3: S16-S20.

The 26583 molecules of the invention are also useful as pharmacogenomic markers. As used herein, a “pharmacogenomic marker” is an objective biochemical marker which correlates with a specific clinical drug response or susceptibility in a subject (see, e.g., McLeod *et al.* (1999) *Eur. J. Cancer* 35:1650-1652). The presence or quantity of the pharmacogenomic marker is related to the predicted response of the subject to a specific drug or class of drugs prior to administration of the drug. By assessing the presence or quantity of one or more pharmacogenomic markers in a subject, a drug therapy which is most appropriate for the subject, or which is predicted to have a greater degree of success, may be selected. For example, based on the presence or quantity of RNA, or protein (e.g., 26583 protein or RNA) for specific tumor markers in a subject, a drug or course of treatment may be selected that is optimized for the treatment of the specific tumor likely to be present in the subject. Similarly, the presence or absence of a specific sequence mutation in 26583 DNA may correlate 26583 drug response. The use of pharmacogenomic markers therefore permits the application of the most appropriate treatment for each subject without having to administer the therapy.

#### Pharmaceutical Compositions

The nucleic acid and polypeptides, fragments thereof, as well as anti-26583 antibodies (also referred to herein as “active compounds”) of the invention can be incorporated into pharmaceutical compositions. Such compositions typically include the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable

carrier. As used herein the language “pharmaceutically acceptable carrier” includes solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms

can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization.

Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying, which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules, *e.g.*, gelatin capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser that contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in

the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD<sub>50</sub> (the dose lethal to 50% of the population) and the ED<sub>50</sub> (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD<sub>50</sub>/ED<sub>50</sub>. Compounds that exhibit high therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to

design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED<sub>50</sub> with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC<sub>50</sub> (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

As defined herein, a therapeutically effective amount of protein or polypeptide (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The protein or polypeptide can be administered one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments.

For antibodies, the preferred dosage is 0.1 mg/kg of body weight (generally 10 mg/kg to 20 mg/kg). If the antibody is to act in the brain, a dosage of 50 mg/kg to 100 mg/kg is usually appropriate. Generally, partially human antibodies and fully human antibodies have a longer half-life within the human body than other

The present invention encompasses agents that modulate expression or activity. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics (*e.g.*, peptoids), amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (*i.e.*, including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

An antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or

cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-

5 dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU),

10 cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

15 The conjugates of the invention can be used for modifying a given biological response. The drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein

20 such as tumor necrosis factor,  $\alpha$ -interferon,  $\beta$ -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophase colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

25 Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or

30 by stereotactic injection (see *e.g.*, Chen *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where

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the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or  
5 dispenser together with instructions for administration.

### Methods of Treatment

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder  
10 associated with aberrant or unwanted 26583 expression or activity. As used herein, the term "treatment" is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter,  
15 remedy, ameliorate, improve or affect the disease, the symptoms of disease or the predisposition toward disease. A therapeutic agent includes, but is not limited to, small molecules, peptides, antibodies, ribozymes and antisense oligonucleotides.

With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained  
20 from the field of pharmacogenomics as described below.

In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant or unwanted 26583 expression or activity, by administering to the subject 26583 or an agent that modulates 26583 expression or at least one 26583 activity. Subjects at risk for a disease that is caused  
25 or contributed to by aberrant or unwanted 26583 expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the 26583 aberrance, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on  
30 the type of 26583 aberrance, for example, a 26583 agonist or 26583 antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

It is possible that some 26583 disorders can be caused, at least in part, by an abnormal level of gene product, or by the presence of a gene product exhibiting abnormal activity. As such, the reduction in the level and/or activity of such gene products would bring about the amelioration of disorder symptoms.

5 As discussed above, successful treatment of 26583 disorders can be brought about by techniques that serve to inhibit the expression or activity of target gene products. For example, compounds, *e.g.*, an agent identified using assays described above, that exhibits negative modulatory activities, can be used in accordance with the invention to prevent and/or ameliorate symptoms of 26583 disorders. Such molecules  
10 can include, but are not limited to peptides, phosphopeptides, small organic or inorganic molecules, or antibodies (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies, and Fab, F(ab')<sub>2</sub> and FAb expression library fragments, scFV molecules, and epitope-binding fragments thereof).

15 Further, antisense and ribozyme molecules that inhibit expression of the target gene can also be used in accordance with the invention to reduce the level of target gene expression, thus effectively reducing the level of target gene activity. Still further, triple helix molecules can be utilized in reducing the level of target gene activity. Antisense, ribozyme and triple helix molecules are discussed above.

20 It is possible that the use of antisense, ribozyme, and/or triple helix molecules to reduce or inhibit mutant gene expression can also reduce or inhibit the transcription (triple helix) and/or translation (antisense, ribozyme) of mRNA produced by normal target gene alleles, such that the concentration of normal target gene product present can be lower than is necessary for a normal phenotype. In such cases, nucleic acid  
25 molecules that encode and express target gene polypeptides exhibiting normal target gene activity can be introduced into cells via gene therapy method. Alternatively, in instances in which the target gene encodes an extracellular protein, it can be preferable to co-administer normal target gene protein into the cell or tissue in order to maintain the requisite level of cellular or tissue target gene activity.

30 Another method by which nucleic acid molecules may be utilized in treating or preventing a disease characterized by 26583 expression is through the use of aptamer molecules specific for 26583 protein. Aptamers are nucleic acid molecules having a tertiary structure that permits them to specifically bind to protein ligands

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(see, *e.g.*, Osborne, *et al.* 1997 *Curr. Opin. Chem Biol.* 1(1): 5-9; and Patel, D.J. 1997 *Curr Opin Chem Biol* Jun;1(1):32-46). Since nucleic acid molecules may in many cases, be more conveniently introduced into target cells than therapeutic protein molecules, aptamers offer a method by which 26583 protein activity may be  
 5 specifically decreased without the introduction of drugs or other molecules which may have pluripotent effects.

Antibodies can be generated that are both specific for target gene products and that reduce target gene product activity. Such antibodies may, therefore, by administered in instances whereby negative modulatory techniques are appropriate for  
 10 the treatment of 26583 disorders. For a description of antibodies, see the Antibody section above.

In circumstances wherein injection of an animal or a human subject with a 26583 protein or epitope for stimulating antibody production is harmful to the subject, it is possible to generate an immune response against 26583 through the use of anti-  
 15 idiotypic antibodies (see, for example, Herlyn, D. 1999 *Ann Med* 31(1):66-78; and Bhattacharya-Chatterjee, M., and Foon, K.A. 1998 *Cancer Treat Res* 94:51-68). If an anti-idiotypic antibody is introduced into a mammal or human subject, it should stimulate the production of anti-anti-idiotypic antibodies, which should be specific to the 26583 protein. Vaccines directed to a disease characterized by 26583 expression  
 20 may also be generated in this fashion.

In instances where the target antigen is intracellular and whole antibodies are used, internalizing antibodies may be preferred. Lipofectin or liposomes can be used to deliver the antibody or a fragment of the Fab region that binds to the target antigen into cells. Where fragments of the antibody are used, the smallest inhibitory fragment  
 25 that binds to the target antigen is preferred. For example, peptides having an amino acid sequence corresponding to the Fv region of the antibody can be used. Alternatively, single chain neutralizing antibodies that bind to intracellular target antigens can also be administered. Such single chain antibodies can be administered, for example, by expressing nucleotide sequences encoding single-chain antibodies  
 30 within the target cell population (see *e.g.*, Marasco *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:7889-7893).

The identified compounds that inhibit target gene expression, synthesis and/or activity can be administered to a patient at therapeutically effective doses to prevent,

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treat or ameliorate 26583 disorders. A therapeutically effective dose refers to that amount of the compound sufficient to result in amelioration of symptoms of the disorders.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD<sub>50</sub> and the ED<sub>50</sub> as described above in the Pharmaceutical Composition section.

Another example of determination of effective dose for an individual is the ability to directly assay levels of “free” and “bound” compound in the serum of the test subject. Such assays may utilize antibody mimics and/or “biosensors” that have been created through molecular imprinting techniques. A compound that is able to modulate 26583 activity is used as a template or “imprinting molecule,” to spatially organize polymerizable monomers prior to their polymerization with catalytic reagents. The subsequent removal of the imprinted molecule leaves a polymer matrix that contains a repeated “negative image” of the compound and is able to selectively rebind the molecule under biological assay conditions. A detailed review of this technique can be seen in Ansell, R. J. *et al* (1996) *Current Opinion in Biotechnology* 7:89-94 and in Shea, K.J. (1994) *Trends in Polymer Science* 2:166-173. Such “imprinted” affinity matrixes are amenable to ligand-binding assays, whereby the immobilized monoclonal antibody component is replaced by an appropriately imprinted matrix. An example of the use of such matrixes in this way can be seen in Vlatakis, G. *et al* (1993) *Nature* 361:645-647. Through the use of isotope-labeling, the “free” concentration of compound which modulates the expression or activity of 26583 can be readily monitored and used in calculations of IC<sub>50</sub>.

Such “imprinted” affinity matrixes can also be designed to include fluorescent groups whose photon-emitting properties measurably change upon local and selective binding of target compound. These changes can be readily assayed in real time using appropriate fiberoptic devices, in turn allowing the dose in a test subject to be quickly optimized based on its individual IC<sub>50</sub>. A rudimentary example of such a “biosensor” is discussed in Kriz, D. *et al* (1995) *Analytical Chemistry* 67:2142-2144.

Another aspect of the invention pertains to methods of modulating 26583 expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell with

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26583 or agent that modulates one or more of the activities of 26583 protein activity associated with the cell. An agent that modulates 26583 protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring target molecule of a 26583 protein (*e.g.*, a 26583 substrate or receptor), a 26583  
5 antibody, a 26583 agonist or antagonist, a peptidomimetic of a 26583 agonist or antagonist, or other small molecule.

In one embodiment, the agent stimulates one or more 26583 activities. Examples of such stimulatory agents include active 26583 protein and a nucleic acid molecule encoding 26583. In another embodiment, the agent inhibits one or more  
10 26583 activities. Examples of such inhibitory agents include antisense 26583 nucleic acid molecules, anti-26583 antibodies, and 26583 inhibitors. These modulatory methods can be performed *in vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease  
15 or disorder characterized by aberrant or unwanted expression or activity of a 26583 protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (*e.g.*, an agent identified by a screening assay described herein), or combination of agents that modulates (*e.g.*, up-regulates or down-regulates) 26583 expression or activity. In another embodiment, the method involves  
20 administering a 26583 protein or nucleic acid molecule as therapy to compensate for reduced, aberrant, or unwanted 26583 expression or activity.

Stimulation of 26583 activity is desirable in situations in which 26583 is abnormally down-regulated and/or in which increased 26583 activity is likely to have a beneficial effect. For example, stimulation of 26583 activity is desirable  
25 in situations in which a 26583 is down-regulated and/or in which increased 26583 activity is likely to have a beneficial effect. Likewise, inhibition of 26583 activity is desirable in situations in which 26583 is abnormally up-regulated and/or in which decreased 26583 activity is likely to have a beneficial effect.

### 30 Pharmacogenomics

The 26583 molecules of the present invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on 26583 activity (*e.g.*, 26583 gene expression) as identified by a screening assay described herein can be

administered to individuals to treat (prophylactically or therapeutically) 26583-  
associated disorders associated with aberrant or unwanted 26583 activity (e.g.,  
hyperproliferative disorders, e.g., cancer). In conjunction with such treatment,  
pharmacogenomics may be considered. "Pharmacogenomics," as used herein, refers  
5 to the application of genomics technologies such as gene sequencing, statistical  
genetics, and gene expression analysis to drugs in clinical development and on the  
market. More specifically, the term refers the study of how a patient's genes  
determine his or her response to a drug (e.g., a patient's "drug response phenotype,"  
or "drug response genotype.") Thus, another aspect of the invention provides  
10 methods for tailoring an individual's prophylactic or therapeutic treatment with either  
the 26583 molecules of the present invention or 26583 modulators according to that  
individual's drug response genotype.

Pharmacogenomics deals with clinically significant hereditary variations in  
the response to drugs due to altered drug disposition and abnormal action in affected  
15 persons. See, for example, Eichelbaum, M. *et al.* (1996) *Clin. Exp. Pharmacol.*  
*Physiol.* 23(10-11) :983-985 and Linder, M.W. *et al.* (1997) *Clin. Chem.* 43(2):254-  
266. In general, two types of pharmacogenetic conditions can be differentiated.  
Genetic conditions transmitted as a single factor altering the way drugs act on the  
body (altered drug action) or genetic conditions transmitted as single factors altering  
20 the way the body acts on drugs (altered drug metabolism). These pharmacogenetic  
conditions can occur either as rare genetic defects or as naturally occurring  
polymorphisms.

Differences in metabolism of therapeutics can lead to severe toxicity or  
therapeutic failure by altering the relation between dose and blood concentration of  
25 the pharmacologically active drug. Thus, a physician or clinician may consider  
applying knowledge obtained in relevant pharmacogenomics studies in determining  
whether to administer a 44576 molecule or 44576 modulator as well as tailoring the  
dosage and/or therapeutic regimen of treatment with a 44576 molecule or 44576  
modulator.

30 One pharmacogenomics approach to identifying genes that predict drug  
response, known as "a genome-wide association," relies primarily on a high-  
resolution map of the human genome consisting of already known gene-related  
markers (e.g., a "bi-allelic" gene marker map which consists of 60,000-100,000

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polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect.

5 Alternatively, such a high-resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a “SNP” is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast  
10 majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

15 Alternatively, a method termed the “candidate gene approach,” can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drug’s target is known (*e.g.*, a 26583 protein of the present invention), all common variants of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with  
20 a particular drug response.

Alternatively, a method termed “gene expression profiling,” can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (*e.g.*, a 26583 molecule or 26583 modulator of the present invention) can give an indication whether gene pathways related to toxicity have been  
25 turned on.

Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment of an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus  
30 enhance therapeutic or prophylactic efficiency when treating a subject with a 26583 molecule or 26583 modulator, such as a modulator identified by one of the exemplary screening assays described herein.

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The present invention further provides methods for identifying new agents, or combinations, that are based on identifying agents that modulate the activity of one or more of the gene products encoded by one or more of the 26583 genes of the present invention, wherein these products may be associated with resistance of the cells to a therapeutic agent. Specifically, the activity of the proteins encoded by the 26583 genes of the present invention can be used as a basis for identifying agents for overcoming agent resistance. By blocking the activity of one or more of the resistance proteins, target cells, e.g., cancer cells, will become sensitive to treatment with an agent that the unmodified target cells were resistant to.

Monitoring the influence of agents (e.g., drugs) on the expression or activity of a 26583 protein can be applied in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase 26583 gene expression, protein levels, or up-regulate 26583 activity, can be monitored in clinical trials of subjects exhibiting decreased 26583 gene expression, protein levels, or down-regulated 26583 activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease 26583 gene expression, protein levels, or down-regulate 26583 activity, can be monitored in clinical trials of subjects exhibiting increased 26583 gene expression, protein levels, or upregulated 26583 activity. In such clinical trials, the expression or activity of a 26583 gene, and preferably, other genes that have been implicated in, for example, a 26583-associated disorder can be used as a “read out” or markers of the phenotype of a particular cell.

#### 26583 Informatics

The sequence of a 26583 molecule is provided in a variety of media to facilitate use thereof. A sequence can be provided as a manufacture, other than an isolated nucleic acid or amino acid molecule, which contains a 26583. Such a manufacture can provide a nucleotide or amino acid sequence, e.g., an open reading frame, in a form which allows examination of the manufacture using means not directly applicable to examining the nucleotide or amino acid sequences, or a subset thereof, as they exists in nature or in purified form. The sequence information can include, but is not limited to, 26583 full-length nucleotide and/or amino acid sequences, partial nucleotide and/or amino acid sequences, polymorphic sequences including single nucleotide polymorphisms (SNPs), epitope sequence, and the like. In



As used herein, “machine-readable media” refers to any medium that can be read and accessed directly by a machine, e.g., a digital computer or analogue

A variety of data storage structures are available to a skilled artisan for creating a machine-readable medium having recorded thereon a nucleotide or amino acid sequence of the present invention. The choice of the data storage structure will generally be based on the means chosen to access the stored information. In addition, a variety of data processor programs and formats can be used to store the nucleotide sequence information of the present invention on computer readable medium. The sequence information can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect and Microsoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like. The skilled artisan can readily adapt any number of data processor structuring formats (*e.g.*, text file or database) in order to obtain computer readable medium having recorded thereon the nucleotide sequence information of the present invention.

In a preferred embodiment, the sequence information is stored in a relational database (such as Sybase or Oracle). The database can have a first table for storing sequence (nucleic acid and/or amino acid sequence) information. The sequence information can be stored in one field (e.g., a first column) of a table row and an identifier for the sequence can be stored in another field (e.g., a second column) of the

table row. The database can have a second table, e.g., storing annotations. The second table can have a field for the sequence identifier, a field for a descriptor or annotation text (e.g., the descriptor can refer to a functionality of the sequence, a field for the initial position in the sequence to which the annotation refers, and a field for the ultimate position in the sequence to which the annotation refers. Non-limiting examples for annotation to nucleic acid sequences include polymorphisms (e.g., SNP's) translational regulatory sites and splice junctions. Non-limiting examples for annotations to amino acid sequence include polypeptide domains, e.g., a domain described herein; active sites and other functional amino acids; and modification sites.

By providing the nucleotide or amino acid sequences of the invention in computer readable form, the skilled artisan can routinely access the sequence information for a variety of purposes. For example, one skilled in the art can use the nucleotide or amino acid sequences of the invention in computer readable form to compare a target sequence or target structural motif with the sequence information stored within the data storage means. A search is used to identify fragments or regions of the sequences of the invention which match a particular target sequence or target motif. The search can be a BLAST search or other routine sequence comparison, e.g., a search described herein.

Thus, in one aspect, the invention features a method of analyzing 26583, e.g., analyzing structure, function, or relatedness to one or more other nucleic acid or amino acid sequences. The method includes: providing a 26583 nucleic acid or amino acid sequence; comparing the 26583 sequence with a second sequence, e.g., one or more preferably a plurality of sequences from a collection of sequences, e.g., a nucleic acid or protein sequence database to thereby analyze 26583. The method can be performed in a machine, e.g., a computer, or manually by a skilled artisan.

The method can include evaluating the sequence identity between a 26583 sequence and a database sequence. The method can be performed by accessing the database at a second site, e.g., over the Internet.

As used herein, a "target sequence" can be any DNA or amino acid sequence of six or more nucleotides or two or more amino acids. A skilled artisan can readily recognize that the longer a target sequence is, the less likely a target sequence will be present as a random occurrence in the database. Typical sequence lengths of a target sequence are from about 10 to 100 amino acids or from about 30 to 300 nucleotide

residues. However, it is well recognized that commercially important fragments, such as sequence fragments involved in gene expression and protein processing, may be of shorter length.

Computer software is publicly available which allows a skilled artisan to  
5 access sequence information provided in a computer readable medium for analysis and comparison to other sequences. A variety of known algorithms are disclosed publicly and a variety of commercially available software for conducting search means are and can be used in the computer-based systems of the present invention. Examples of such software include, but are not limited to, MacPattern (EMBL),  
10 BLASTN and BLASTX (NCBI).

Thus, the invention features a method of making a computer readable record of a sequence of a 26583 sequence which includes recording the sequence on a computer readable matrix. In a preferred embodiment the record includes one or more of the following: identification of an ORF; identification of a domain, region, or  
15 site; identification of the start of transcription; identification of the transcription terminator; the full length amino acid sequence of the protein, or a mature form thereof; the 5' end of the translated region.

In another aspect, the invention features, a method of analyzing a sequence. The method includes: providing a 26583 sequence, or record, in machine-readable  
20 form; comparing a second sequence to the 26583 sequence; thereby analyzing a sequence. Comparison can include comparing to sequences for sequence identity or determining if one sequence is included within the other, e.g., determining if the 26583 sequence includes a sequence being compared. In a preferred embodiment the 26583 or second sequence is stored on a first computer, e.g., at a first site and the  
25 comparison is performed, read, or recorded on a second computer, e.g., at a second site. E.g., the 26583 or second sequence can be stored in a public or proprietary database in one computer, and the results of the comparison performed, read, or recorded on a second computer. In a preferred embodiment the record includes one or more of the following: identification of an ORF; identification of a domain, region, or  
30 site; identification of the start of transcription; identification of the transcription terminator; the full length amino acid sequence of the protein, or a mature form thereof; the 5' end of the translated region.

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In another aspect, the invention provides a machine-readable medium for holding instructions for performing a method for determining whether a subject has a 26583-associated disease or disorder or a pre-disposition to a 26583-associated disease or disorder, wherein the method comprises the steps of determining 26583 sequence information associated with the subject and based on the 26583 sequence information, determining whether the subject has a 26583-associated disease or disorder or a pre-disposition to a 26583-associated disease or disorder and/or recommending a particular treatment for the disease, disorder or pre-disease condition.

The invention further provides in an electronic system and/or in a network, a method for determining whether a subject has a 26583-associated disease or disorder or a pre-disposition to a disease associated with a 26583 wherein the method comprises the steps of determining 26583 sequence information associated with the subject, and based on the 26583 sequence information, determining whether the subject has a 26583-associated disease or disorder or a pre-disposition to a 26583-associated disease or disorder, and/or recommending a particular treatment for the disease, disorder or pre-disease condition. In a preferred embodiment, the method further includes the step of receiving information, e.g., phenotypic or genotypic information, associated with the subject and/or acquiring from a network phenotypic information associated with the subject. The information can be stored in a database, e.g., a relational database. In another embodiment, the method further includes accessing the database, e.g., for records relating to other subjects, comparing the 26583 sequence of the subject to the 26583 sequences in the database to thereby determine whether the subject as a 26583-associated disease or disorder, or a pre-disposition for such.

The present invention also provides in a network, a method for determining whether a subject has a 26583 associated disease or disorder or a pre-disposition to a 26583-associated disease or disorder associated with 26583, said method comprising the steps of receiving 26583 sequence information from the subject and/or information related thereto, receiving phenotypic information associated with the subject, acquiring information from the network corresponding to 26583 and/or \$ corresponding to a 26583-associated disease or disorder (e.g., a metabolic disorder, e.g., a mitochondrial related disorder or a cholesterol biosynthesis related disorder; or

a cellular proliferation and/or differentiation disorder), and based on one or more of the phenotypic information, the 26583 information (*e.g.*, sequence information and/or information related thereto), and the acquired information, determining whether the subject has a 26583-associated disease or disorder or a pre-disposition to a 26583-associated disease or disorder. The method may further comprise the step of recommending a particular treatment for the disease, disorder or pre-disease condition.

The present invention also provides a method for determining whether a subject has a 26583 -associated disease or disorder or a pre-disposition to a 26583-associated disease or disorder, said method comprising the steps of receiving information related to 26583 (*e.g.*, sequence information and/or information related thereto), receiving phenotypic information associated with the subject, acquiring information from the network related to 26583 and/or related to a 26583-associated disease or disorder, and based on one or more of the phenotypic information, the 26583 information, and the acquired information, determining whether the subject has a 26583-associated disease or disorder or a pre-disposition to a 26583-associated disease or disorder. The method may further comprise the step of recommending a particular treatment for the disease, disorder or pre-disease condition.

This invention is further illustrated by the following examples that should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are incorporated herein by reference.

### EXAMPLES

#### Example 1: Identification and Characterization of Human 26583 cDNA

The human 26583 sequence (Figure 1; SEQ ID NO:1), which is approximately 2838 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 1613 nucleotides (nucleotides 462 to 2075 of SEQ ID NO:1; SEQ ID NO:3). The coding sequence encodes a 537 amino acid protein (SEQ ID NO:2).

Example 2: Tissue Distribution of 26583 mRNA

Endogenous human 26583 gene expression was determined using the Perkin-Elmer/ABI 7700 Sequence Detection System which employs TaqMan technology. Briefly, TaqMan technology relies on standard RT-PCR with the addition of a third gene-specific oligonucleotide (referred to as a probe) which has a fluorescent dye coupled to its 5' end (typically 6-FAM) and a quenching dye at the 3' end (typically TAMRA). When the fluorescently tagged oligonucleotide is intact, the fluorescent signal from the 5' dye is quenched. As PCR proceeds, the 5' to 3' nucleolytic activity of Taq polymerase digests the labeled primer, producing a free nucleotide labeled with 6-FAM, which is now detected as a fluorescent signal. The PCR cycle where fluorescence is first released and detected is directly proportional to the starting amount of the gene of interest in the test sample, thus providing a way of quantitating the initial template concentration. Samples can be internally controlled by the addition of a second set of primers/probe specific for a housekeeping gene such as GAPDH which has been labeled with a different fluorophore on the 5' end (typically VIC).

To determine the level of 26583 in various human tissues a primer/probe set was designed using Primer Express (Perkin-Elmer) software and primary cDNA sequence information. Total RNA was prepared from a series of human tissues using an RNeasy kit from Qiagen. First strand cDNA was prepared from 1 µg total RNA using an oligo-dT primer and Superscript II reverse transcriptase (Gibco/BRL). cDNA obtained from approximately 50 ng total RNA was used per TaqMan reaction.

26583 mRNA was analyzed in a variety of normal and tumor clinical tissue samples. 26583 expression was found in human breast, lung, colon, liver, and brain. Figure 4 shows relative 26583 mRNA expression on mRNA derived from the following tissue samples: columns 1-3, normal breast; columns 4-10, breast tumor; columns 11-13, normal lung; columns 14-20, lung tumor; columns 21-23, normal colon; columns 24-31, colon tumor; columns 32-35, colon metastases; columns 36-37, normal liver; columns 38-39, normal brain; columns 40-42, brain tumor. On average, 26583 expression was increased in lung tumor tissue as compared to normal lung tissue. 26583 expression levels were substantially lower in brain tumor tissue compared to normal brain.

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initiation codon; the 3' end sequence contains complementary sequences to the other restriction site of interest, a translation stop codon, the HA tag or FLAG tag and the last 20 nucleotides of the 26583 coding sequence. The PCR amplified fragment and the pCDNA/Amp vector are digested with the appropriate restriction enzymes and the  
 5 vector is dephosphorylated using the CIAP enzyme (New England Biolabs, Beverly, MA). Preferably the two restriction sites chosen are different so that the 26583 gene is inserted in the correct orientation. The ligation mixture is transformed into *E. coli* cells (strains HB101, DH5 $\alpha$ , SURE, available from Stratagene Cloning Systems, La Jolla, CA, can be used), the transformed culture is plated on ampicillin media plates,  
 10 and resistant colonies are selected. Plasmid DNA is isolated from transformants and examined by restriction analysis for the presence of the correct fragment.

COS cells are subsequently transfected with the 26583-pcDNA/Amp plasmid DNA using the calcium phosphate or calcium chloride co-precipitation methods, DEAE-dextran-mediated transfection, lipofection, or electroporation. Other suitable  
 15 methods for transfecting host cells can be found in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989. The expression of the 26583 polypeptide is detected by radiolabeling ( $^{35}\text{S}$ -methionine or  $^{35}\text{S}$ -cysteine available from NEN, Boston, MA, can be used) and  
 20 immunoprecipitation (Harlow, E. and Lane, D. *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988) using an HA specific monoclonal antibody. Briefly, the cells are labeled for 8 hours with  $^{35}\text{S}$ -methionine (or  $^{35}\text{S}$ -cysteine). The culture media are then collected and the cells are lysed using detergents (RIPA buffer, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5%  
 25 DOC, 50 mM Tris, pH 7.5). Both the cell lysate and the culture media are precipitated with an HA specific monoclonal antibody. Precipitated polypeptides are then analyzed by SDS-PAGE.

Alternatively, DNA containing the 26583 coding sequence is cloned directly into the polylinker of the pCDNA/Amp vector using the appropriate restriction sites.  
 30 The resulting plasmid is transfected into COS cells in the manner described above, and the expression of the 26583 polypeptide is detected by radiolabelling and immunoprecipitation using a 26583 specific monoclonal antibody.

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Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

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